A REËXAMINATION OF THE CYTOLOGY OF HYDRACTINIA AND PENNARIA.[1]

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The present report does not claim to be a complete discussion of the cytological phenomena in the developmental history of *Hydractinia* and *Pennaria*, nevertheless, it adds considerable new data hitherto unpublished concerning the problem of the structure of the egg, the migration of the chromatin, maturation, fertilization and mitosis. However, the present paper cannot claim to settle the controversy as to the existence of amitosis because the results concerning which there may be some differences of opinion have been negatively interpreted.

During the summer of 1906 Professor C. W. Hargitt asked me to undertake a reëxamination of some of his work on hydroids paying particular attention to the cytological phenomena of the early development, that being the point of most interest. The results of Bunting, '94, on *Hydractinia* were not in agreement with his observations on *Pennaria*, *Eudendrium*, *Clava*, etc., so that he suggested that it might be well to restudy this form also. While I have had opportunity to examine all of Professor Hargitt's preparations on the several hydroids studied by him, it seems wise to confine this paper to *Hydractinia* and *Pennaria*.

It is not often that such a study as this is undertaken and when it is there is involved a great deal of work that it would be superfluous to republish so that the present paper needs to be read in connection with Bunting, '94, on *Hydractinia* and Hargitt, '04, on *Pennaria*. At the beginning of this study, Professor Hargitt explicitly stated that he wished to give me absolute freedom in the problem and the interpretation of the results. This he has done even to the extent of not seeing any of my preparations until I turned over the finished paper to him.

When he asked me to undertake this restudy, he volunteered to collect and preserve the material necessary. In this particular

[1] Contributions from the Zoölogical Laboratory, Syracuse University, C. W. Hargitt, director.

too much credit cannot be given Professor Hargitt for his persistent experiments in trying to find a suitable fixing reagent for these refractory eggs of hydroids. Had I been without the benefit of his long experience, I doubt if the present results could have been secured. He used, among fixing agents, Bouin's fluid which has given the best fixation of any thus far tried and certainly these preparations are superior in regard to their fixation to any which he made during the preceding years of study. There is very little doubt that the eggs of hydroids degenerate when left in alcohol for some time and should be embedded in paraffin as soon as possible.

It seems unnecessary to review the general literature on this subject for this has already been well done by Hargitt in his numerous papers and by Bigelow, '08. Professor Hargitt is so well known as an authority on hydroids that it has seemed unnecessary for me to make an elaborate critique of his papers. Therefore, I have in most instances simply referred to the pages where he discussed similar phenomena, elaborating only those points upon which I have additional data.

Origin of the Eggs in *Hydractinia.*

The eggs arise in the entoderm close to the basement membrane. Certain entoderm cells are directly transformed without any immediately preceding cell division. These young ova are distinguished by having a large nucleus and granular cytoplasm. No special region in the polyp is devoted to the production of ova as Bunting, '94, maintains and text-figures 1 and 2 demonstrate. The young ova are as likely to begin their growth in the cells in the base of the polyp as in those along the side.

Growth of the Egg in *Hydractinia.*

The first apparent change in these entoderm cells which are to become ova that has thus far been observed is a marked increase in the size of the nucleus before the cell as a whole has undergone any change. The result is that the nucleus occupies most of the cell, the cytoplasm being limited to a narrow border. A comparison of the nuclear contents at this early stage with the surrounding entoderm cells shows that the nucleolus is increas-

ing rapidly in size. In the entoderm cells it is so very small that it is difficult to be sure of its presence in some cells, and the same is true in some of the young ova, but in most instances it is a clearly defined and easily distinguished body.

The most interesting change is found in the chromatin network. In the young ova it is now in its most conspicuous state. From this time on until new ova arise in a new polyp the chromatin does not possess such distinctness. A loose network is distributed through the nucleus with conspicuous masses where the threads cross. The achromatic substance (residual substance of Lillie, '06) does not stain in the acid or basic stains. The nuclear membrane is very distinct, chiefly due to the fact that a considerable amount of chromatin material seems to be directly in contact with it. The cytoplasm is as yet free from the microsomes — minute granules — which characterize the slightly older ova.

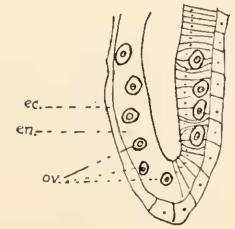

FIG. 1. Outline drawing of base of polyp to show the position of the ova. *ec*, ectoderm; *en*, entoderm; *ov*, ova. *Hydractinia*.

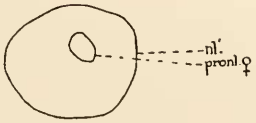

FIG. 3. Camera lucida drawing of the egg nucleus, the larger circle, and the female pronucleus, the smaller circle. *Nl*, nucleus; pronl. ♀, female pronucleus.

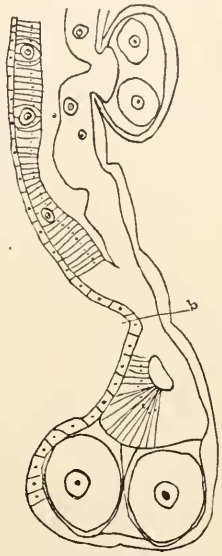

FIG. 2. Outline drawing to show that sometimes the Hydractinian polyp branches. *B*, base of polyp. Reconstructed from several sections.

The next step in this gradual transformation is the rapid increase in the cytoplasm, accompanied by a growth in the nucleus. The cytoplasm is now loosely sprinkled with microsomes, in

places giving the appearance of a reticulum. With the accumulation of the microsomes, the reticulate condition is obliterated and the cytoplasm becomes a dense mass of microsomes. In such young ova the achromatic substance of the nucleus takes a plasma stain such as Orange G or Bordeaux red. The vacuolation of the nucleolus has begun by this time, the vacuoles taking the plasma stain. This vacuolation of the nucleolus is in agreement with the many cases already described. The vacuolation continues until the nucleolus disappears which occurs before maturation. The growth, vacuolation and eventual disappearance of the nucleolus is an event which takes place during the growth of the egg, but is not synchronous with any definite phase of this growth.

The young ova thus described are found in the entoderm at the base, the side of the polyp, or in the gonophore. About this time the ova take their permanent position (Bunting) in the gonophore, *i. e.*, in the ectoderm where they increase greatly in size. This increase in size is largely a matter associated with the growth of the individual microsomes into small spherules. We are led to believe from this study that there are also many microsomes added which likewise are changed into spherules. These spherules are so numerous in the adult egg as to conceal the ultimate structure of the cytoplasm. They take a dense stain but not always a homogeneous one. During this gradual growth, the staining reaction changes, a change which can be readily seen on a slide where all stages are represented. A number of such were studied where all of the material had received the same treatment from fixation to and including staining. On such a slide the young ova are so deeply stained as to conceal most of the details of structure while the large eggs show but a faint response to the stain. But when the more mature eggs are once stained in iron hæmatoxylin, it takes considerable time to differentiate them in the iron so tenaciously do they hold the stain. Under these conditions one may see on the same slide young ova black and blue-black in color while the older eggs are hardly stained at all. Between these two extremes, there are all gradations. This change in color reaction is evidently due to the transformation of the microsomes into spherules.

Chromatin Changes in the Egg of *Hydractinia*.

While the cytoplasm is becoming thus transformed, the nucleus has increased in size although it does not become more distinct. The nucleolus is mostly occupied by a large vacuole. In place of the sharply defined chromatin masses in the younger ova, there is a marked change in this particular. The typical chromatin reaction is hardly evident and the whole nucleus tends to take a stain similar to the cytoplasm. The chromatin threads and masses are less definite in position and arrangement. The question naturally arises, what is becoming of the chromatin? Can its disappearance be traced into any part of the egg? In other words, is there a definite and specific migration of chromatin from the nucleus into the cytoplasm? The following facts are submitted in answer to these queries.

While the eggs are still in the gonophore and the nucleolus is becoming vacuolated, small particles of chromatin leave the nucleus and wander out into the cytoplasm. Fig. 1, Pl. I., shows the early stages of this process, some of the chromatin granules are just emerging. Others have moved some distance. My attention was first directed to this phenomenon by finding eggs which showed conditions such as Fig. 2, Pl. I., typifies. A number of densely staining chromatin granules are scattered in the cytoplasm and mostly surrounded by a narrow clear area. These masses of chromatin are small and usually single but occasionally two or three are found in a single vacuole. The reason for regarding these masses as chromatin is because they give the same color reaction as similar shaped bodies in the nucleus; and for the further reason which is obvious in Fig. 1, namely, the actual migration of the chromatin from the nucleus.

When the size of the nucleus of the mature egg, *i. e.*, before maturation, is compared with the female pro-nucleus one is strongly impressed with the great reduction in size. Text-fig. 3 is a camera lucida drawing of the outline of the egg nucleus as represented by the larger circle, while the smaller circle within represents the size of the female pro-nucleus. It must be apparent at once that up to and during maturation there is a remarkable reduction in the size of the nucleus which the maturation phenomenon alone does not adequately explain. The nucleus

loses a large amount of chromatin by direct migration into the cytoplasm which is entirely independent of the chromatin discharged during the formation of the polar bodies. The subsequent fate of this discharged chromatin has been studied with much pains and it is the belief of the writer that it is something as follows: When the cytoplasm of the egg is examined after it has been discharged from the gonophore there appear many areas that are free from the characteristic granules of the surrounding cytoplasm. These areas are usually round and contain small particles that stain with Borax carmine or hæmatoxylin. They look so much like faintly staining nuclei that their appearance is very confusing at first (Fig. 6). As segmentation progresses, these areas tend to migrate to the periphery of the egg and are occasionally so numerous that they form a nearly continuous row around the embryo, Fig. 9. Eventually they are absorbed by the cytoplasm. This explanation, then, traces the highly vacuolated condition of the cytoplasm in *Hydractinia* directly to the migration of chromatin from the nucleus before maturation begins. A similar series of changes occurs in *Pennaria* but at a different time.

Localization of the Formative Stuffs in *Hydractinia*.

The following extract indicates the extent of the previous description of the structure of the cytoplasm. "Sections of the egg show deutoplasm spheres distributed throughout the protoplasm, with the exception of the outer rim which is composed entirely of protoplasm" (Bunting, page 215).

The cytoplasm exhibits a rather definite localization of the so-called formative stuffs in the presence of a coarsely granular crescentic area (picro-acetic fixation) located on the side of the egg in which the nucleus lies — the animal pole. The appearance of these granules in *Hydractinia* is very similar to what Hargitt, '06, p. 214, has found in *Clava*. In addition to these bodies there are some minute bodies located around the periphery of the egg in a narrow band which takes a deep blue stain (Borax carmine, Lyons blue method). These particles do not stain readily and so were overlooked by Bunting. By the regular hæmotoxylin methods they are usually indistinguishable from the other micro-

somes and spherules. This gives three distinct bodies in the cytoplasm: (1) The ordinary bodies all through the cytoplasm and usually interpreted as yolk masses; (2) the coarse granules distributed in crescentic bands; (3) the small bodies around the periphery. The small peripheral granules remain on the outside of the embryo during cleavage and can be traced into the planula. In the planula they are confined to the ectoderm. The differentiation and subsequent fate of similar particles has been made out in *Pennaria*. The first and second class of granules are chiefly confined to the mass of cells within the ectoderm of the planula, although a few scattering granules are seen in the ectoderm.

Maturation in *Hydractinia*.

The following quotation reveals the extent of previous observations on this phase of development. "The ovum while in the gonophore has a well-defined nucleus situated above the center of the egg, which fades from view when the ovum is deposited" (Bunting, page 215). "In about fifteen minutes after the ova are laid the polar bodies appear. When first observed two globules were present, one had been extruded, while the other one was just appearing. One of the two divided subsequently, in a plane at right angles to the first cleavage plane of the ovum. Within ten minutes from the extrusion of the first polar body, the second was ejected" (Bunting, page 216).

The nucleus during growth varies from round to elliptical and in this latter shape the ends may nearly reach to the periphery but until maturation begins, the nucleus is central in position. It is to be regretted that more stages in maturation have not been discovered notwithstanding the fact that large numbers of slides have been made of eggs just after deposition. Maturation begins before the eggs are discharged from the gonophore but just how long I am unable to state. The fact that this process begins while the eggs are still retained, makes the solving of the problem tedious in as much as the gonophores are not set free as are the medusæ in *Pennaria*. Occasionally a gonophore containing eggs is found among the recently discharged eggs and it was in such that the first signs of maturation were detected. A large number of slides were made of the large gonophores from colonies that

were laying eggs when fixed but none of these showed any of the maturation phenomena. Fig. 3, Pl. I., shows the prophase of the first maturation. The asters have moved part way around the nucleus and a few spindle fibers are evident. The nuclear wall on the side toward the middle has been partly broken down. The chromatin shows but a slight tendency to take a stain although it is collecting into definite masses. The chief importance of this drawing centers around the process by means of which maturation occurs, namely, the mitotic process.

When this material was collected it was not thought that maturation began until after deposition because of the observations quoted. For that reason much time was spent studying the eggs just after deposition, but in all cases none of the earlier stages were found. In two or three eggs undoubted polar bodies were found after the eggs had been deposited, and such a case is shown in Fig. 4, Pl. I. Fig. 4 represents the late telophase of what I judge to be the second maturation. In the polar cell there are several vesicles which probably represent chromatin. In the egg the chromatin vessels are very small and grouped among the remains of the breaking down astral fibers. These vesicles collect into a single vesicle, the female pro-nucleus, Fig. 5, Pl. I.

Certainly these two figures do not furnish a complete account of maturation, but they do show: (1) The nature of the process; (2) that this process begins in the gonophore; (3) that Miss Bunting's observations are not correct, because if what she observed were polar bodies they would be found attached to the egg before and after segmentation, but such is not the case. The polar bodies usually drop off as soon as the eggs are discharged. In all of the eggs studied but two were found which retained the egg nucleus after the egg had been deposited.

One of the curious conditions is shown in Fig. 5, where the female pro-nucleus lies next to the periphery of the egg with a few fibers apparently starting from its outer pole. This was thought for some time to mean the prophase in maturation, but never could fibers or an aster be found at the inner pole. Just why there should be a small furrow over this nucleus I do not know, and such a furrow does not always occur. It might be

thought to mean the beginning of segmentation, but such a furrow may appear before fertilization takes place. In a number of instances the nucleus in this same stage was seen to be partly protruding from the substance of the cytoplasm, a condition for which no explanation is offered. The chromatin in the female pro-nucleus takes a faint stain up to the time of the first cleavage. The differentiation of the egg of *Hydractinia* is very difficult, much more so than in *Pennaria*, which makes the recognition of these very arduous. In the region of the nucleus in Fig. 5 there are several deeply staining particles which look much like chromatin, but of their nature I am uncertain. That this nucleus is the female pro-nucleus the following reasons indicate: (1) The absence of the nucleolus; (2) its relatively small size; (3) that it has been traced directly into the first cleavage.

Fertilization in *Hydractinia*.

Thus far no sperms have been found in the eggs before they were deposited but after deposition spermatozoa are seen in contact with the eggs. The sperm head becomes transformed into a vesicle soon after it penetrates the cytoplasm. There does not seem to be any definite place where the sperm enters the egg. During the progress of the male pro-nucleus through the cytoplasm, no aster was seen nor any definite path. The staining reaction of this body is so very faint that it is made out only after careful study with the oil immersion.

Fig. 6, Pl. I., shows the approach of the male pro-nucleus and the change in shape of the female pro-nucleus preparatory to the prophase of the first segmentation. No asters or radiations could be distinguished in connection with either of these pro-nuclei at this stage. These observations further show that the egg nucleus does not "fade from view when the ovum is deposited" but that it can be traced continuously from the egg in the gonophore to the first segmentation stage.

Cleavage in *Hydractinia*.

The difficulties encountered in differentiating these eggs has made it almost impossible to discover a complete series of the changes in any of the stages as many of the mitotic phenomena

are visible only with the oil immersion lens. This means that but few eggs will be cut in just the right plane to enable one to make out the correct relations ; and it also means that important conditions will escape detection.

The first division of the egg into the two-cell stage is preceded by the formation of a definite mitotic figure after the male and female pro-nuclei have come together. The chromatin becomes more responsive to stain and gradually condenses into a definite number of very minute chromosomes. These chromosomes in size and staining reaction are so similar to some of the granules in the cytoplasm, Fig. 8, Pl. II., that it is impossible to be certain that they are chromosomes unless the spindle fibers are present. This makes the determination of the number of chromosomes difficult because when one has a cross-section of the metaphase through the equatorial region, one cannot determine the relation of the spindle fibers to the chromosomes and so cannot be certain of their number. The task was a little easier in *Pennaria*, where fourteen were counted in the anaphase, although here I am not certain that this is the correct number. I think that there are from twelve to sixteen chromosomes present in these hydroids, the exact number remains to be determined.

The chromosomes form in the typical metaphase condition, split and move toward each pole of the spindle. In the anaphase distinct interzonal fibers are present. During the telophase the chromosomes are transformed into a nucleus. This nucleus does not necessarily assume the rounded outline but is often elongated and even irregular in shape. The prophase of the next cleavage frequently begins while the nucleus is in this condition, Fig. 7, Pl. I. In Fig. 7 a typical prophase of mitosis in cleavage is shown. The faint asters are on opposite sides of the elongate nucleus, and a few spindle fibers are forming preparatory to the metaphase and the dissolution of the nucleus. The centrosphere as shown in Figs. 7 and 8 will be discussed in a separate section.

A definite mitotic figure has been made out in all of the early stages of segmentation and followed up to the planula stage. A typical condition of the early embryo is shown in outline in Fig. 9, Pl. II. The cells surround a cavity which is first seen in the two-cell stage and is due to the separation of these first two cells.

This cavity increases in extent as segmentation continues. After a time the peripheral cells begin to segment in such a manner as to set cells free in this cavity. The direction of the spindle in Fig. 8 shows the method. Fig. 8 was taken from one of the cells shown in Fig. 9 and in each of the remaining blastomeres there is a mitotic figure, so placed as to give rise to a cell that becomes free in the segmentation cavity. The segmentation cavity finally becomes full of cells due to the setting free of cells from the periphery and the subsequent division of these same cells as they lie in this cavity.

Membranes in *Pennaria*.

First or False Membrane. — The absence of a membrane or membrane-like structure in the animal egg is doubted by some notwithstanding that Wilson, '82, Metschnikoff, '86, Hargitt, '04, and others have repeatedly stated that the eggs of hydroids are naked. Brauer, '91, finds in *Hydra*, after the embryo is formed, two membranes produced by the ectoderm, but the unsegmented egg is naked. Previous studies in *Pennaria* make no mention of an egg membrane, but the ectosarcal portion of the egg is described by Hargitt as forming at times a filamentous membrane around the cytoplasmic papillæ. Aside from these references no mention is made of membrane in hydroids.

When the eggs of *Pennaria* are well fixed in Bouin, and a hæmatoxylin stain is followed by a plasma stain such as Bordeaux red, a rather broad but uneven structure is readily observed. It first forms while the egg is still growing and is easily made out while the eggs are still in the medusa. The portion of the egg adjacent to the manubrium shows pseudopodia-like processes (cf. Hargitt) and between these processes this structure is quite wide and irregular in width. After the eggs are deposited and have assumed the rounded outline, this membrane-like structure appears as shown in text-fig. 4. It is rarely of uniform thickness and usually shows one place that is bulging. Also

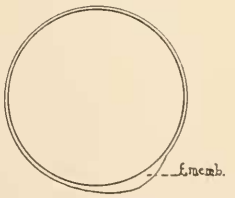

Fig. 4. Outline drawing to show the relative thickness and variability in width of the false membrane. *F. memb*, false membrane.

in the laid eggs as segmentation begins and continues, it appears torn so that some of the eggs will be only partly surrounded by it. Some time during the progress of cleavage this membrane disappears entirely. It is well known that the newly deposited eggs of *Pennaria* are inclined to stick to bits of grass, a glass dish, etc., which is due to the adhesive property of the false membrane. The term false membrane is applied to this structure because it is not permanent; and the term membrane is used because it seems to serve the purpose and do the work of a real membrane. No differential lamellæ were visible in this false membrane either before or after deposition. Numerous sperm heads are frequently to be distinguished within its substance. The inequalities in thickness of the false membrane suggest that this substance is of a fluid nature in the living egg and transparent. If this interpretation prevails, then one can no longer speak of the eggs in *Pennaria* as being naked.

Second or True Membrane. — After fertilization and with the beginning of cleavage a second membrane begins to form (cf. Hargitt) which lies in close contact with the granules of the cytoplasm and completely surrounds each cell as it is produced in cleavage. This structure compares favorably in appearance, staining reaction, etc., with the regularly described egg membranes of animal eggs and so is designated as the true membrane.

Maturation of the Egg in *Pennaria*.

From the early development of the egg up to the beginning of maturation I cannot add any new data, but do confirm Hargitt's ('04, p. 456) observations. The extensive observations of Hargitt as well as my present independent studies indicate that it is very rare to find a polar body attached to the egg after deposition, not one in one hundred will show a polar body at this time. It is also very rare to find a deposited egg that still retains the egg nucleus with a nucleolus. In fact this latter structure, the nucleolus, has been taken as an indication of whether maturation has taken place or not. Where it is lacking I have designated the nucleus as the female pro-nucleus. The egg nucleus in *Pennaria* does not show any such great size as is found in *Hydractinia* and varies but slightly from the female pro-nucleus which is

always present in the egg previous to the beginning of segmentation.

Sections of medusæ after they have become free from the colony and before the eggs are discharged may show the presence of polar bodies. Several different slides show conditions such as are indicated in Fig. 10, Pl. II. The first polar body is being shoved to one side by the formation of the second. The chromatin is in the form of three vesicles in the first polar body. Even while the egg is in the medusa, the polar body may be pushed some distance from the spot where it emerged which may be due to the contractions of the bell of the medusa. The second polar body has ten vesicles in two groups ; while thirteen are made out deeper in the egg. To the right there is one isolated vesicle. This one and several of the thirteen contain granules of chromatin. This interpretation of the vesicles grows out of my

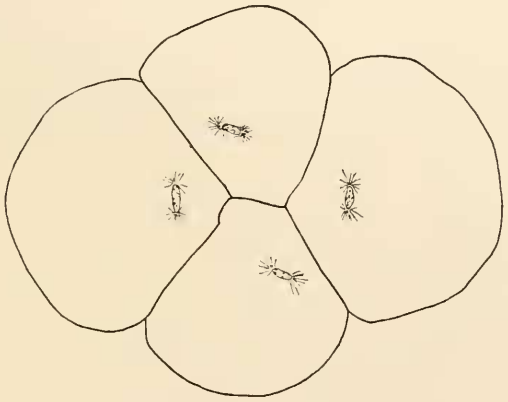

FIG. 5. A regular segmentation stage, all cells in prophase.

study of the changes through which the chromatin passes during cleavage. The several vesicles deep in the egg unite into a single definite nucleus like the one in Fig. 11, Pl. II. That these polar bodies are formed by the mitotic process, there is but little doubt because of similar conditions in *Hydractinia* where there can be no doubt as to the beginning of the process ; and because of the state of the chromatin, which in this condition of several vesicles, is entirely unlike the amitotic process of division.

In the same medusa from which Fig. 10 was taken there were

three other eggs in the same phase of maturation. *Pennaria* was collected by Professor Hargitt early in the morning and in the afternoon but in the numerous sections made of the adult medusæ, none showed any earlier stages. With this data at hand, however, it should be an easy matter to secure the intervening stages which undoubtedly occur about the time the medusæ are set free. Sections of medusæ still contained one or two eggs which in each instance had completed maturation, Fig. 11, and the polar bodies were not in contact with the egg. The conditions shown in Figs. 47 and 49 by Hargitt are without question polar bodies and probably the second. The state of the chromatin vesicles in Fig. 47 is more regular than any that I have seen which may be due to the preservation. They seem also to be more scattered than any observed in eggs fixed in Bouin. The clear area within the egg just beneath these vesicles was not seen in the Bouin fixation. Another puzzling feature of this study on maturation was the presence of small bodies attached to the surface of the egg. They were especially noticeable on the eggs of *Hydractinia*. After some study, it was apparent that they were protozoa which in many instances looked exactly like polar cells found in mollusca.

Chromatin Changes in *Pennaria*.

The female pro-nucleus is a small, faintly staining body that is found in eggs that are just laid and many that are still in the medusa. Sometimes it was pointed at the outer end and pushed close against the false membrane but it was never found protruding. It occupies this position until the approach of the male pro-nucleus when it may move some distance from the periphery of the egg. But before the fusion of the two pro-nuclei, there occurs a series of unusual changes in the chromatin, especially of the female pro-nucleus. Changes of a similar nature but not as extensive are found taking place in the male pro-nucleus. The chromatin changes described in *Hydractinia* preceded maturation. These in *Pennaria* follow maturation.

It is difficult to determine whether there is any order to these changes and so no attempt is made to decide which is the older state in the series of Figs. 12 to 18. In several of these figures

the male pro-nucleus is shown but its position, near or far from the female pro-nucleus does not seem to influence the time when the chromatin is to migrate from the nucleus. Immediately in contact with each pro-nucleus, the cytoplasm becomes denser and is composed of finer granules (cf. also Hargitt, Figs. 48, 49, 50). But isolated or wandering nuclear vesicles usually lack this modification of the cytoplasm which has been used to assist in recognizing the pro-nuclei.

Figs. 12, Pl. II., 15 and 18, Pl. III., show some of the chromatin granules close to the nuclear membrane as if they had just emerged from the nucleus into the cytoplasm. In both Figs. 15 and 18 these chromatin granules are on opposite sides of the nucleus which indicates that their position is not the result of the accident of cutting the sections. The male pro-nucleus in Fig. 15 shows the same condition of the chromatin. After the chromatin has been in the cytoplasm for some time, there are found definite small vesicles usually empty. From the study of mitosis in cleavage and the changes in the chromatin during the anaphase and telophase, the suggestion that these vesicles are the product of the transformed chromatin seems inevitable. During this period, while the chromatin is migrating into the cytoplasm, the chromatin both within and without the nucleus takes a very faint stain so that the whole nucleus is easily overlooked. Some of the most satisfactory results were obtained by using Brazilian without the iron mordant. In no instance have nuclei entirely devoid of chromatin been found. The vesicles in Figs. 13, 14, 17, Pl. II., and most of them in Fig. 12, are empty. If the interpretation offered is correct, then there must be a very large amount of chromatin that leaves the nucleus in Fig. 12. The meaning of the large flask-shaped vesicle attached to the female pro-nucleus in Fig. 15 is not understood.

There is some question as to whether this process takes place in all of the eggs preparatory to cleavage, but that it is very common and appears in well fixed eggs there can be no question. On the same slides were found mitotic figures preserved in all of their parts. The slides showing many of these phenomena were examined by Mr. George T. Hargitt who is at work on a similar problem at Harvard University and he confirmed the correctness

of these observations. The conditions certainly exist as drawn but more drawings could be made easily showing different relations of the chromatin masses and vesicles. The eggs just after deposition do not show these chromatin changes. After a time the chromatin that remains in the male and female pro-nuclei increases in amount and takes a deeper stain until the chromatin appears like that shown in Figs. 16, Pl. IV., and 19, Pl. III. Hargitt in Fig. 48 has two similar vesicles.

The vesicles last but a short time and usually are indistinguishable by the time that segmentation begins. But when such conditions as shown in Fig. 16 exist in the presence of a small vesicle containing chromatin near the female pro-nucleus and a second one between the two pro-nuclei, these remain longer in the egg. What their influence on subsequent development is, some light may be gained by a study of Fig. 49 (Hargitt). In this drawing there is a clearly defined mitotic figure with faint chromosomes; and a short distance away, three asters and their connecting fibers are around several vesicles. This Fig. 49 seems to be a later stage in the transformation of some of these vesicles containing chromatin and indicates a pseudo-segmentation in that it is not preceded by the fusion of the male and female pro-nuclei. It hardly seems as if such conditions played an important part in the future segmentation.

Fertilization in the Egg of *Pennaria*.

The spermatazoa penetrates the egg of *Pennaria* frequently before deposition, but the penetration is usual after the egg is laid. The false membrane may contain a large number of sperm heads that were unable to gain admission into the egg. The sperm head immediately after penetration becomes transformed into a vesiculate body, the male pro-nucleus. No definite place of entrance, nor path through the cytoplasm, nor the presence of asters could be determined. It remains smaller than the female pro-nucleus and is surrounded by fine cytoplasmic granules. Figs. 15, Pl. IV., 16, Pl. III., and 18, Pl. IV., give a good idea of the male pro-nucleus.

Figs. 17 and 19 show the approach of the pro-nuclei. The fine cytoplasmic granules surrounding the two bodies have united

in Figs. 15 and 19 and may have a stellate outline. It was practically impossible in Fig. 19 to determine whether there was a fusion at this stage of the two nuclei or whether they were merely in contact.

Polyspermia. — In Fig. 20, Pl. IV., there are shown conditions which seem to point strongly to polyspermia. The female pro-nucleus is present and some distance away a single vesicle which is similar in every respect to the male pro-nucleus of later stages. The cytoplasm is indented just opposite as if modified by the recent penetration of the sperm. Then close by this vesicle are nine other vesicles, somewhat smaller but otherwise identical with the single one. There is likewise the same modification of the cytoplasm just opposite. The natural explanation seems to be to regard these all as transformed sperm heads, and consequently polyspermia. Fig. 21, Pl. III., shows a second case of probable polyspermia where there are three nuclei, one much larger which is probably the female pro-nucleus. The other two are interpreted as male pro-nuclei. That several nuclei may exist previous to the first segmentation like this Fig. 21 and Fig. 48 of Hargitt there can be no question and that they are in some instances due to polyspermia I feel equally certain. But that this is the exclusive interpretation I do not accept because nuclear division may outrun cytoplasmic cleavage which results in giving several nuclei in the cytoplasm as Figs. 43 and 43*a* of Hargitt's clearly indicate and I have confirmed. It seems more probable that both explanations should be used in interpreting the multiple nuclear conditions.

Cleavage of the Egg in *Pennaria.*

This part of the paper on cleavage tends to show the manner of cleavage rather than a detailed description of the process as this has already been done by Hargitt. As was described in the section on fertilization, the two pro-nuclei approach preparatory to the first cleavage. Fig. 22, Pl. II., shows the prophase of the first cleavage. The female pro-nucleus has become much elongated and there are distinct asters at each end. The spindle fibers are just forming and the chromatin is still in the loose stage ; the chromosomes are yet to form. The male pro-nucleus,

such of it as appears in the section, is near one end of the female pro-nucleus and much smaller. In Fig. 23 is shown another form of the prophase. This drawing was taken from the beginning of the second cleavage. The small prophase spindle lies entirely within the centrosphere and the chromosomes are in the form of vesicles. That these vesicles are modified chromosomes is proved by the condition of the chromosomes at the opposite pole of the old spindle where the process was not as advanced. Here some of the interzonal fibers were still in contact with the partly transformed chromosomes. The successive cleavage is so rapid in this instance that the new spindle has formed before the vesicles have united into a single vesicle, the nucleus. Nevertheless a normal spindle will result when the chromosome vesicles are transformed again into chromosomes. It is the study of such changes as these in the chromatin that convinced me that the vesicles in Fig. 10 were modified chromosomes and the vesicles in Figs. 12–16 were formed through the influence of chromatin astray in the cytoplasm. A third form of the prophase is quite common, Fig. 24, Pl. IV., where the nucleus is much elongated or somewhat irregular in outline. This prophase stage is farther advanced than the two previously described. The asters are larger and the forming spindle fibers more pronounced at each end. The chromatin has begun to take a deeper stain. In some respects all of these three forms of prophase are different, but the differences are not fundamental, and plenty of similar variations are known in other animals. They are all unmistakably by the mitotic process.

The metaphase is as typical as exists in any mitotically dividing cell. The chromosomes split and move to each pole, Figs. 23, 26, 27, Pl. II. During the late anaphase and early telophase the chromosomes become transformed into vesicles, Figs. 27, 23, may or may not unite into a single vesiculate nucleus before the next cleavage. I have been able to trace the nucleus continuously from its state in the unmaturated egg through all of the cleavage states. At no times does it dissolve other than in the normal mitotic changes. At no time does the total contents of the nucleus become dissipated throughout the cytoplasm to reform into separate nuclei. Other than the chromatin changes just

previous to segmentation, the nuclear phenomena in *Pennaria* appear perfectly normal.

Sometimes the cleavage is very regular as text-fig. 5 indicates, each cell shows the nucleus in the prophase and apparently so placed as to give off cells in the clockwise directions. The tilting of these nuclei and the perfect regularity is so apparent that this drawing might have been taken from an annelid or mollusc-an embryo, but in this same group of cells there was a most irregular embryo so that this regular condition is nothing more than an accident and is the only regular embryo among many sections.

The Centrosphere in the Egg of *Pennaria*.

It is difficult to decide on any of the old terms to describe the conditions in *Pennaria*. If the term centrosphere may include sphere and centrosome, the latter being potentially present only, then this may be taken as an acceptable term.

If there is a centrosome present in these eggs then it is an unstable body which varies greatly and is recognized with much difficulty. In all cases of the prophase, there remains a small clear area from which the fibers radiate but into which they do not penetrate. Fig. 24, Pl. III., shows a few fine granules in this area but their size, number, and staining reaction does not indicate that they are at all constant, nor can one detect that there is any inherent relation between these granules and rays or spindle fibers. That there is probably some substance that plays the part of a centrosome is apparent in Fig. 23, Pl. III., where the whole new spindle lies entirely within the old centrosphere. Therefore, it seems as if one might say that certain of these granules have potential centrosome properties and are possibly in the way to become differentiated; that is to say, that the elaborate centrosomes of mollusca for example indicate a higher degree of differentiation, while in these hydroids a similar result is produced by several granules which cannot be differentiated from the rest of the sphere substance.

The transformation of this centrosphere keeps pace with the changes in the chromosomes. By the time that the anaphase is reached the centrosphere substance has increased greatly in size,

Figs. 25, 26; and in late anaphase is very conspicuous, Fig. 27. After the metaphase stage, the astral fibers are mostly composed of granules, Figs. 25, 26. The interzonal fibers persist for some time and are frequently bent as the cleaving cytoplasm passes through them. At such times, the chromosomes lie at each end of a V-shaped figure. As the fibers disappear and the chromosomes become transformed into a nucleus, the centrosphere substance largely becomes an indistinguishable part of the cytoplasm, but a small portion remains as a clear, narrow region surrounding the nucleus. This means that the deeply staining, newly formed "resting nucleus" with a narrow transparent area around it in cleavage is perfectly normal, being the last remains of the centrosphere.

Papillæ in *Hydractinia* and *Pennaria*.

Hargitt (p. 469) has described these as ectosarcal phenomena. My study confirms his and shows that in both of these hydroids the papillæ are common in the unsegmented egg and have even been seen while the egg was in the medusa. There is no particular region on the egg where they arise. At first these papillæ were regarded as polar bodies, especially in *Hydractinia*, but when they were found on the vegetal pole and the female pro-nucleus was present at the animal pole in the same egg, such an interpretation was impossible. I believe that these papillæ are what Miss Bunting saw and described as polar cells, which is a mistake that might be easily made. In *Pennaria* these papillæ push the false membrane away from the egg as they form and after being set free remain in this same structure for some time. The papillæ in both species are wholly devoid of chromatin and so far as could be determined entirely cytoplasmic phenomena.

Fragmentation of the Nucleus, — Amitosis.

Fragmentation. — If by fragmentation of the nucleus is meant that the entire nucleus disappears and its contents disperse throughout the cytoplasm then I find no evidence of such a process in these hydroids. But what shall be said of the chromatin changes before maturation in *Hydractinia* and in *Pennaria* after maturation where large quantities of chromatin migrate into

the cytoplasm never to return to the nucleus so far as one can determine? This certainly seems to be a kind of fragmentation. Why does it occur? Has the maturation phenomena failed to fully prepare the egg nucleus for fertilization? These are questions which those who would make the development of all eggs conform to a definite series of changes must explain.

Amitosis. — By this process it is understood that the nucleus divides without the chromatin passing through a complicated series of changes and without the formation of a spindle. Frequent search has been made for amitosis in these eggs but without finding any positive evidence. The irregular and claviculate shaped nuclei were critically observed and in every instance eventually either asters or the very characteristic chromatin changes were taking place in them. The mere shape of the nucleus in *Pennaria* is no indication of amitosis, nor is it necessary that the chromosome vesicles become transformed into the single "resting nucleus." *The cluster of vesicles which Hargitt frequently finds is not uncommon in my material but is interpreted in this paper as late telophase.* This is a point concerning which there may be a difference of opinion but taking all of the facts into consideration, these vesicles seem to me to indicate mitosis rather than amitosis.

Inclusions in *Pennaria.*

There are found in the eggs of *Pennaria,* even before maturation, bodies which for the lack of a better term are designated inclusions, Fig. 29, Pl. IV. Thus far they have not been seen in the segmenting egg. As many as three such inclusions have been discovered in a single egg. When newly formed, the substance of the inclusion takes the same stain as the surrounding cytoplasm, but in the older stages this contained substance stains faintly, eventually leaving a cavity. This cavity then is obliterated by the encroachment of the cytoplasm. The substance within the inclusions in appearance and staining reactions is certainly similar, if not identical, to the cytoplasm, and the whole structure looks like a food-vacuole in which cytoplasm is being digested. Their origin has not been determined. They can hardly be regarded as polar bodies, because they may appear before maturation begins.

Summary.

1. In many particulars this work is a confirmation of the previous paper by Hargitt, especially in regard to the several nuclei in the unsegmented egg, the irregular shape of many such nuclei, and the irregular phases of cleavage.

2. The ova arise in any region of the polyp, which is contrary to Bunting's statements. The young ova gradually increase in size, during which time the nucleolus becomes vacuolated and the cytoplasm is occupied by numerous microsomes which become transformed into spherules. The cytoplasm changes its staining reaction during this time.

3. The chromatin during the growth of the egg stains less intensely than when in the younger state. Much of the chromatin migrates into the cytoplasm and is surrounded by vacuoles. The highly vacuolated condition of the cytoplasm is probably directly due to this migrating chromatin. The size of the nucleus decreases greatly.

4. In *Hydractinia* there were found three distinct kinds of granules, yolk masses, coarse granules and small bodies around the periphery. The small granules are distributed exclusively to the ectoderms.

5. Maturation begins in *Hydractinia* before the eggs are deposited. The process is by the formation of a distinct mitotic figure. It is very rare to find a polar body attached to the deposited egg.

6. The female pro-nucleus is very much smaller than the egg nucleus before maturation but it persists as a definite structure until cleavage begins. It is not at any time indistinguishable. The male pro-nucleus moves through the cytoplasm until it approaches the female pro-nucleus when the two fuse and fertilization is effected.

7. The first, and all subsequent cleavages, is by the mitotic process. A definite segmentation cavity is formed in the two-celled stage which increases in size. This cavity is gradually filled with cells until the planula is a solid mass of cells.

8. The false membrane in *Pennaria* is a transitory structure and probably of a fluid nature. Later its place is taken by a true membrane.

9. Maturation in *Pennaria* begins before the eggs leave the medusa. The polar bodies are ephemeral in character and rarely found attached to the deposited egg. The polar bodies are formed by the mitotic process.

10. After the two polar bodies are formed in *Pennaria* there is a distinct migration of a considerable amount of chromatin into the cytoplasm. The chromatin is transformed into vesicles which eventually are taken up by the cytoplasm. Sometimes these vesicles contain chromatin and persist for some time and may (?) divide mitotically, giving rise to a pseudosegmentation.

11. The spermatozoa may penetrate the egg before it is laid. The sperm head is transformed into a male pro-nucleus which moves through the cytoplasm toward the animal pole to unite with the female pro-nucleus.

12. Cleavage in *Pennaria* is at all times by the mitotic process. The chromosomes become transformed into vesicles during the late anaphase and early telophase. The vesicles may or may not unite into a definite "resting nucleus" before the next cleavage.

13. During cleavage in *Pennaria* there is a distinct centrosphere which contains granules with centrosome powers. This centrosphere is more conspicuous in *Pennaria* than in *Hydractinia*. The new prophase spindle arises within the old centrosphere.

14. Papillæ are found in both *Hydractinia* and *Pennaria* before segmentation, much as described by Hargitt.

15. A partial condition of fragmentation is seen in the migration of chromatin into the cytoplasm in both species.

16. No clear evidence of amitosis was observed.

17. Inclusions are frequently found in the egg of *Pennaria*.

October 20, 1908.

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POSTSCRIPT.

CHAS. W. HARGITT.

As supplemental to the foregoing paper it may not be amiss to add a few brief notes and comments. First, to the effect that it comprises an integral phase of work which has engaged the writer for many years, and which is still in progress. This particular feature was undertaken at my solicitation in the summer of 1906, as stated by the author. Second, at the same time I likewise turned over to my son, G. T. Hargitt, material for work along similar lines, a brief report of which has already been made. (*Science*, March 12, 1909.)

Smallwood's paper was completed nearly a year ago, but was at my request delayed, pending completion of further work of my own which was well advanced, and which it was intended should appear at the same time as intimately related thereto. The appearance of a brief note by Beckwith (Biol. Bull., March, 1909), suggests the desirability of the issue of Smallwood's paper without further delay.

Aside from one or two points suggested by Beckwith I shall not undertake any detailed comments in this connection. Her rather matter-of-course dismissal of my presumed errors as to maturation with the remark that it was due "simply to the fact that eggs were not collected at the right time of day" is, to say the least, somewhat gratuitous! One does not usually follow in-

vestigations over a period of ten years without having taken *some* precautions against the more obvious sources of error. The fact is, I had long ago provided against that contingency. Again, her equally hasty dismissal of any question of methods of technique is without warrant. It was this more than any other one matter that proved an obstacle to satisfactory cytologic results. This I have called attention to in at least two of my more recent papers. And the precaution mentioned in the above paper by Smallwood as to this point was explicitly my own suggestion.

A brief comment as to the question of amitosis raised by both Smallwood and Beckwith must suffice for the *present.* In the first place I have never questioned the fact of mitosis in any of the cases under review, as the most cursory attention to my papers will show. Whether there be *amitosis* is purely a question of fact. Were my own results the only evidence it might very well be questioned. Facts adduced from almost every phylum of the animal kingdom are too well known at present to warrant further dogmatism on *a priori* or theoretical grounds. Whether my interpretation of the significance of the nuclear and chromatin fragmentation and the vesiculate "nuclear nests" may be warranted I shall defer for later consideration.

EXPLANATION OF PLATES.

Explanation of Plate I.

Fig. 1. Egg nucleus with small amount of cytoplasm to show the migration of the chromatin. *N'*, nucleus; *N''*, nucleolus; *chr*, chromatin.

Fig. 2. Portion of the cytoplasm and nucleus. The chromatin is mostly in vacuoles. *N'*, nucleus; *chr*, chromatin.

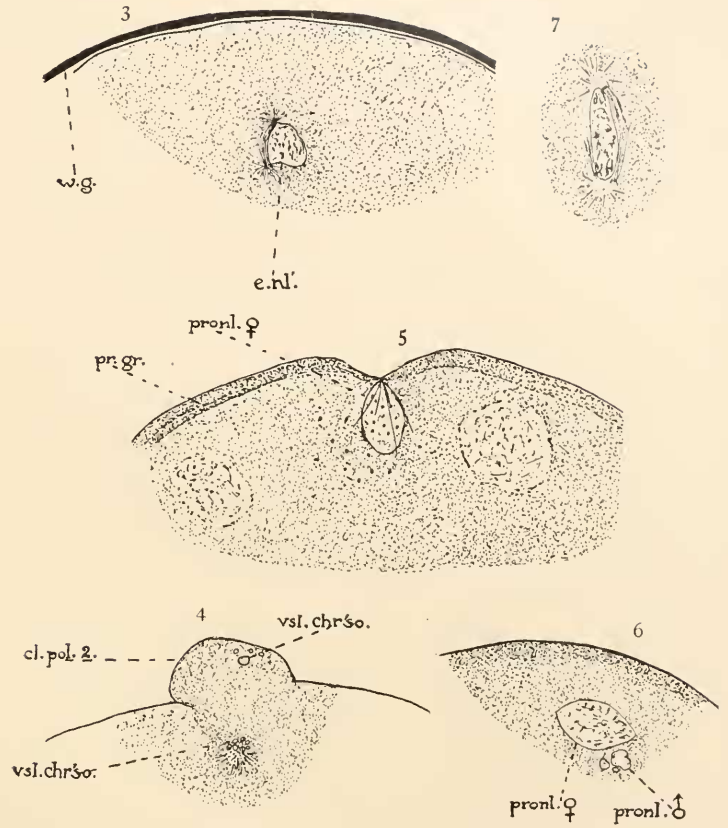
Fig. 3. Prophase first maturation. The walls of the nucleus are still intact. The egg is within the gonophore. *E. N'*, egg nucleus; *w. g*, wall of gonophore.

Fig. 4. Telophase of the second maturation. Egg deposited. *Cl. pol*^{2}, second polar body; *vsl. chr'so*, chromosome vesicles.

Fig. 5. Female pronucleus showing the remains of fibers that have persisted. Pronl. ♀, female pronucleus. *pr. gr*, peripheral granules.

Fig. 6. The approach of the male pronucleus. The female pronucleus is in the early prophase. Pronl. ♀, female pronucleus; pronl. ♂, male pronucleus.

Fig. 7. Prophase of third cleavage shows an elongated nucleus with asters and forming spindle fibers.


3
w.g.
e.nl'.
7
pronl. ♀
pr. gr.
5
4
vsl. chrśo.
cl. pol. 2.
vsl. chrśo.
6
pronl. ♀
pronl. ♂
1
nl'.
nll.
ch
2
chr.
nl'

Explanation of Plate II.

Fig. 8. Is taken from one of the cells shown in Fig. 9. The vacuolated condition of the cytoplasm is shown in the clear spaces. The spindle is in the anaphase and so directed as to set a cell free in the segmentation cavity. There is a large centrosphere at each pole.

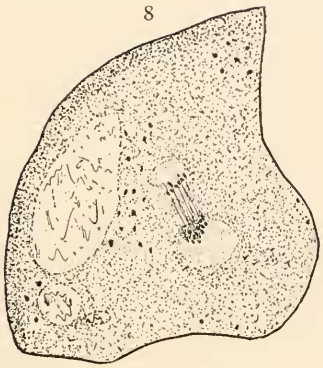
Fig. 9. Outline drawings of embryo. Numerous vacuoles are present in the cells. *Seg. cav*, segmentation cavity.

Figs. 10 to 29 are from *Pennaria*.

Fig. 10. Telophase second maturation. Egg in medusa. *Cl. pol. 1*, first polar body; *cl. pol. 2*, second polar body.

Fig. 11. Female pronucleus drawn from an egg before deposition. Pronl. ♀, female pronucleus.

Figs. 12 to 18 show the migration of the chromatin from the pronuclei into the cytoplasm. Pronl. ♀, female pronucleus; ♂, male pronucleus.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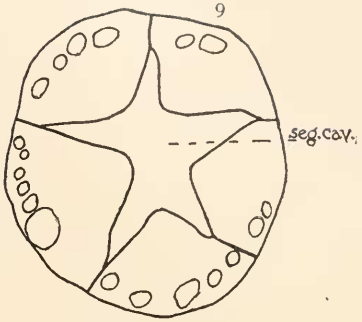
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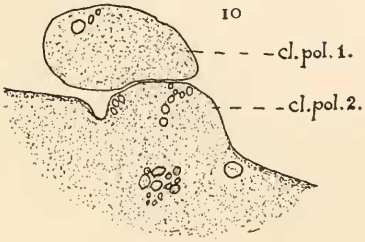
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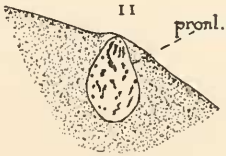
cl.pol.2.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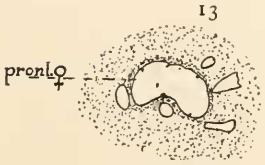
pronl.♀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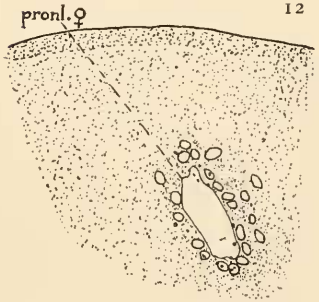
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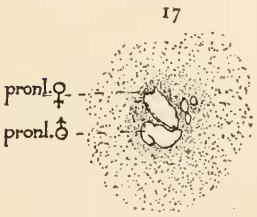
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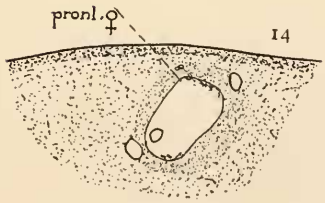
pronl.♀
pronl.♂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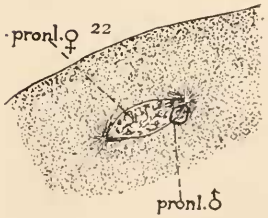
pronl.♀
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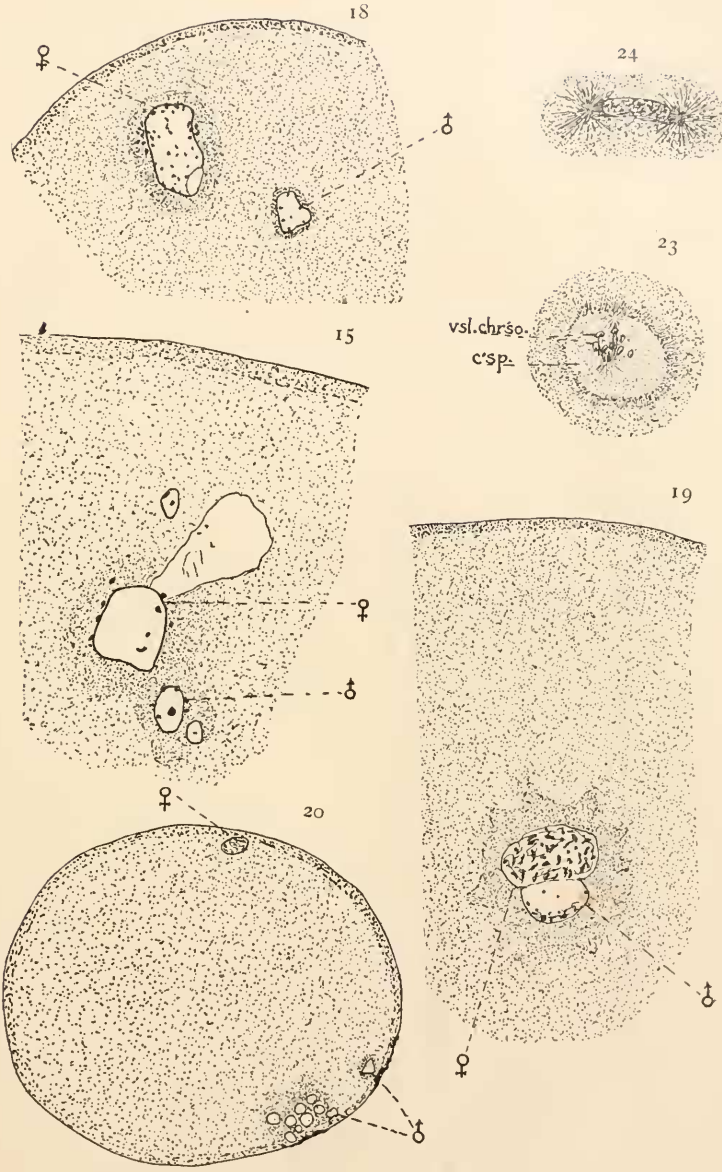
pronl.♂

Explanation of Plate III.

Fig. 19. Union of the male and female pronuclei. Pronl. ♀, female pronucleus; pronl. ♂, male pronucleus.

Fig. 20. Low power drawing of entire egg. The fine peripheral granules extend clear around the egg. Several nuclear-like bodies are interpreted as polyspermia.


18
♀
♂
24
23
vsl.chrso.
c'sp.
15
19
♀
♂
20
♀
♂
♀
♂

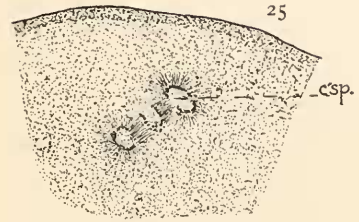
Explanation of Plate IV.

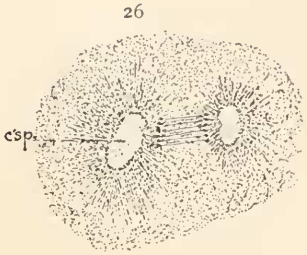
Fig. 21. Shows the presence of several nuclei in the unsegmented egg, one of which is probably the female pronucleus, pronl. ♀.

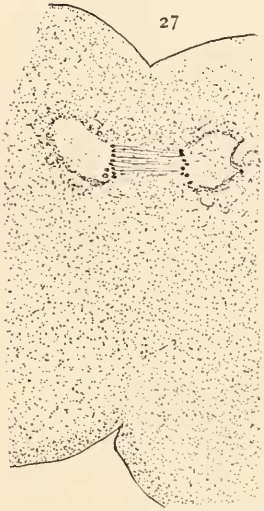
Figs. 22 to 24. Three forms of the prophase in cleavage. Fig. 22, conjugation of pronuclei and formation of first segmentation spindle. In Fig. 23 the chromosome vesicles have not united. Pronl. ♀, female pronucleus; pronl. ♂, male pronucleus; *vsl. chr'so*, chromosome vesicles; *c'sph*, centrosphere.

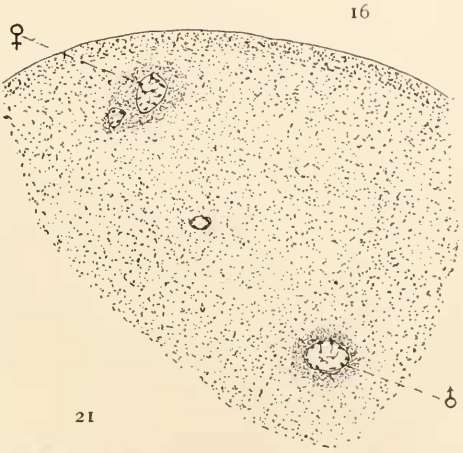
Figs. 25, 26, 27. Show the appearance of the segmentation spindle, migration of the chromosomes, and presence of the centrosphere; *c'sph*, centrosphere.

Fig. 28. Inclusions in the cytoplasm.









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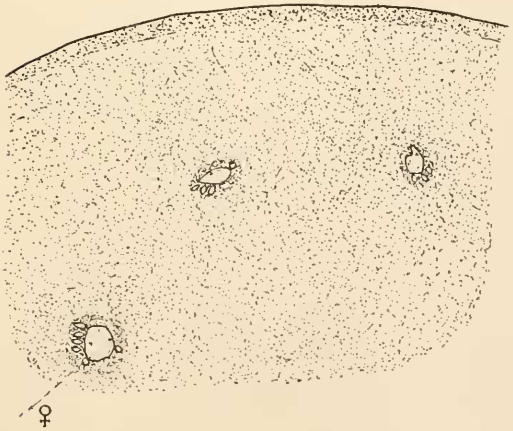

NEW OR LITTLE KNOWN PERMIAN VERTEBRATES. PARIOTICHUS.[1]

S. W. WILLISTON,

University of Chicago.

The University of Chicago Expedition to the Permian of Texas during the autumn of 1908 was very fortunate in finding a skeleton of a small reptile enclosed in a nodular matrix, probably the most complete of any specimen hitherto obtained from that formation. It is of especial interest since it gives, for the first time, a natural skeleton of a cotylosaur with all its bones in anatomical relations, scarcely a single one disturbed by extraneous force in fossilization. The original nodule measured about six inches in its greater, by five in its lesser diameter, and about two inches in thickness. The nodule, as discovered, was split horizontally, the thicker portion enclosing most of the skeleton lying upon its back; the thinner with portions of the bones partly enclosed in it, and with the right front leg almost wholly so. One small piece of the thicker side, and a yet smaller fragment of the thinner were not recovered. The missing portions, however, are of minor importance, and are in part supplemented by the two blocks. The specimen was discovered on a gently sloping surface near the Wichita River, north of Mabelle, by Mr. Paul Miller.

The material of which the nodule is composed is a rather hard argillaceous limestone, and has necessitated very patient labor on the part of Mr. Miller, with awl and needle, in its preparation, many of the bones being so small as to require the use of a magnifying glass. The skeleton, which measures nearly fifteen inches in length, is closely coiled, the tip of the tail lying under the front extremity of the skull. As exposed on the two blocks, the hyoid bones are in place; the pectoral girdle is very slightly displaced, with both arms articulated; the right arm is strongly flexed at the elbow, with its outspread hand underlying the pos-

[1] "Cotylosauria," *Journal of Geology*, XVI., p. 139; *Lysorophus*, this journal, XV., p. 229; *Diplocaulus*, *Trans. Kansas Acad. Science* (in press); *Trematops*, *Journal of Geology*, XVII. (in press).

terior part of the right mandible; the left arm is extended backward close to the vertebral column, its fingers protruding from the edge of the block and for the most part lost; both hind legs

FIG. 1. *Pariotichus laticeps* Williston. Photograph of specimen, from the thicker half of nodule; natural size.

are articulated throughout, turned backward by the side of the tail, and not a single bone seems to have been lost or disarticu-

lated ; both the feet, unfortunately, are lying in part upon their fibular side, concealing some of the bones ; the tenth to the thirteenth caudal vertebræ are disarranged and partly missing, probably due to the fact that they lie partly over the right foot ; the small terminal vertebræ of the tail are also missing, where

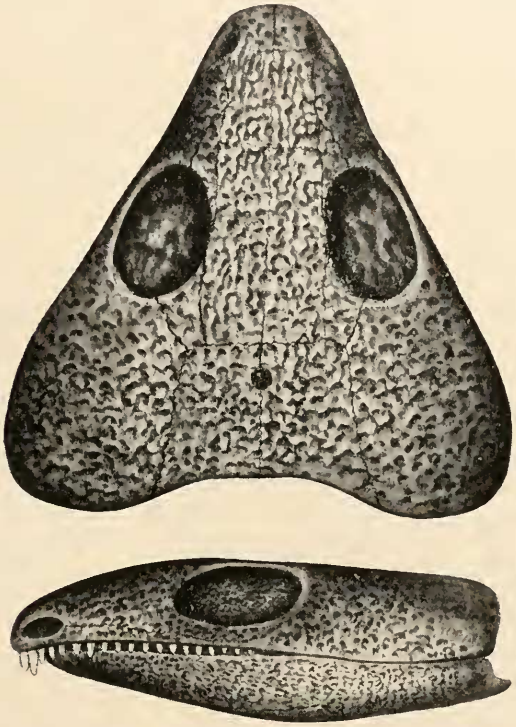

FIG. 2. *Pariotichus laticeps*, skull from above and from the side ; natural size.

they protruded from the margin of the slightly eroded nodule. On the whole, the only doubtful details of the skeleton are the number and arrangement of the tarsal bones, and the extreme tip of the tail. The extreme terminal phalanges of the first three fingers, because of their minute size, may have been destroyed in the preparation of the block, or slightly dislodged.

Skull (Figs. 1, 2). — The skull is in a marvellously perfect condition, the only injury it has suffered being a very slight erosion of the extreme tip of the muzzle where it protruded from the edge of the nodule. It is remarkable among reptiles for its

great width and depression, its width posteriorly being fully equal to its length. The pitting of the upper surface is small and reticulate, with a slight, though distinct, indication of longitudinal ridges. The parietal and frontal bones posteriorly are slightly concave in the middle. The nares are small, situated nearly at the extremity of the muzzle, oval in shape and directed more outward. In front of the orbits the face is a little constricted, with the sides more strongly convex. From the front of the orbits the lateral lobe of the cranium is convex outward to about midway between the orbit and the hind border, where the curve is slightly inward. There is a rather strong emargination of the hinder border of the cranium in the middle, with the outer third on each side gently convex or nearly straight. The large orbits are located a trifle in front of the middle of the skull. They are oval in outline, with the long diameter antero-posterior, measuring eighteen millimeters in length by fifteen in breadth. The plane of their margins is directed a little forward and upward at an angle of about 45°. The large pineal foramen is nearly midway between a line drawn through the posterior margin of the orbits and the hind border of the skull in the middle line. The sutures of the skull are, for the most part, indicated by very delicate lines, requiring a hand lens to follow. I have given such as I feel sure of. The plane of the upper surface of the skull is nearly horizontal as far forward as the front end of the orbits, whence it slopes gently to the front extremity, with a slight convexity. The mandibles are in position on the under side, slightly pushed to the left. The symphysis is short, the rami rather narrow in front, their outlines very much like those of the side of the cranium, curving inward posteriorly. They are broadest and deepest a little back of their middle, or opposite the transverse bones, which abut against them. Distinct sutures for the splenial, dentary and angular are seen anteriorly on the outer side. The dentary ends by a broad curve upward, with the slender prolongation of the angular enclosed between it and the margin of the splenial below. At the hind extremity the small, inwardly curved angular process is visible. The slight lateral pressure upon the mandibles has left exposed the insertion of the maxillary teeth of the right side, but the very delicate teeth them-

selves have suffered in the preparation, though their roots are clearly to be seen. On the premaxillary there are three stout teeth, the largest of the series of either jaw. Of these the third is the smallest, the first and second of nearly equal size, judging from their roots. Back of the premaxillary teeth, in addition to the roots seen on the right side, the teeth themselves are preserved on the left side, crowded rather closely upon the mandible. They are all rather small, the fifth or sixth of the series, counting the premaxillary teeth, about a third of the distance to the orbit back of the narial opening, is the largest. They are all rather obtusely pointed, and are separated by spaces about equal to or somewhat less than the width of the teeth themselves. In a space of ten millimeters back of the largest maxillary tooth there are five teeth. The mandibular teeth cannot be made out. It is altogether probable that there are additional teeth in secondary rows upon the maxilla and dentary, but the close union of the mandibles with the cranium prevents their detection.

The palate is very fully exposed and is undistorted. The internal nares, placed far forward, are above the mandibles and yet concealed by the matrix. The broad, flat palatines and vomers — for sutures are nowhere determinable — diverge very gradually to about midway between the mandibular symphysis and the basisphenoid, where they separate more widely, leaving a rather large ovate space, yet filled in with matrix. I can distinguish no presphenoid in this ovate space, but it is possible one exists directed obliquely toward the roof of the skull. Along the margin of these bones by the side of the ovate opening and anteriorly are one or two rows of minute tubercular teeth, and, just in front of the descending convexity of the transverse, there is an oblique row of six or seven palatine teeth on each side. The lateral palatal plates descend posteriorly in a convex surface, to nearly the lower margin of the mandibles, with a thinned, convex posterior margin. The lower convexity of these transverse bones (for they are doubtless separate elements, though they have never been suturally distinguished) is covered with a patch of tubercular teeth. The union of the pterygoids with the basisphenoid is very evident in the constriction at either side of the front of the basisphenoid, but the suture is not determinable. From this con-

striction and union with the short basipterygoid processes of the basisphenoid, the posterior plates of the pterygoids diverge to the inner side of the quadrates, opposite the inner angle of the articular bone of the mandible. These plates have a thin, straight, horizontal lower margin, whence they slope inwardly and upwardly into a somewhat concave surface, narrow and nearly vertical in front, widened behind, leaving a small oval space anteriorly between them and the basisphenoid. The basisphenoid is narrow in front, gently widened behind, shallowly concave in the middle and limited on either side by a rather rugose ridge. From near the posterior, somewhat divergent, ends of these ridges, a slender bone runs outward and backward by the side of the inner margin of the pterygoid plate. It is in all probability the stapes. Upon the whole, the structure of the palate and occipital region is quite like that of *L. hamatus*, which will be fully described and illustrated in a future paper from a remarkably perfect specimen in the University collections. Lying under the palate were two slender rods. One of these has been necessarily destroyed in getting to the palatal surface; the other still remains in the matrix. They nearly meet in the middle anteriorly, just back of the interpterygoid vacuity, diverging posteriorly nearly parallel with the margins of the pterygoid plates to near the quadrate. Back of these and articulated with them are a pair of more slender bones bent inwardly near their middle, and terminating acutely behind, nearly parallel with the front margins of the clavicles.

Vertebræ. — The vertebræ are united in a continuous series from the skull to near the tip of the tail, forming a U-shaped curve. The pectoral girdle still conceals the anterior five or six of these and the posterior seven of the presacral series were lost in the missing fragment of the nodule. However, the top of the nodule over these missing seven vertebræ has preserved in part impressions of them, and, inasmuch as the vertebræ themselves, where exposed, are all of precisely the same length, seven and a half or eight millimeters, the number of presacral vertebræ in the entire series is determinable with but slight chance of error. This number was either twenty-three or twenty-four. Broili has determined the number of presacral vertebræ in *Labidosaurus hamatus* as either twenty-four or twenty-five; twenty-three or possibly

twenty-four is the number of presacral vertebræ in the rhachitomous *Trematops* as determined by me ; twenty-three or twenty-four was the number ascertained by Thevenin in *Sauravus costei*, a probable reptile from the Upper Carboniferous of France ; and I believe that *Isodectes Copei*, from the Lower or Middle Mississippian of Linton, Ohio, had the same number. This uniformity seems to be more than a coincidence. Of this number the atlas alone can be called, as in the amphibians, a true cervical. All the vetebræ bore ribs, and, inasmuch as there was no sternum in these early forms, a distinction into neck and trunk is impossible. The centra of our specimen are rather slender — more so than in *Labidosaurus* — each with a marked constriction in the middle. Some of the posterior vertebræ lie partly upon their sides, disclosing the attachment of the ribs. No distinct process is seen on the centrum for them, differing in this respect from *Labidosaurus*, though doubtless the lower part of the proximal extremity of the ribs did articulate with the centrum. A small intercentrum is present in one or two of the anterior vertebræ, and a small space is seen between the lower edges of the centra of all. The sacral vertebræ are of course not visible in the pelvis. There were, in all probability, but two sacral vertebræ, though this is not certain since another form of Permian reptile, which will be illustrated and described later, has three, while *Labidosaurus* has but two. If the vertebræ above the pubes and ischia are of the same length as those immediately following or preceding, there are six concealed from view, one of which is the first presacral, and three the basal caudals. Behind the pelvis eight caudal vertebræ in a connected, gently curved series are visible. They are somewhat shorter and more slender than the presacral vertebræ, the entire series measuring about forty-six millimeters. Beginning with the second of these there are long, slender chevrons, each reaching to beyond the end of the next succeeding centrum, that is, about fourteen millimeters in length. Just how far back these chevrons continue is not certain, but at least eight centra bore them. The first entire vertebra back of the pelvis bears a long, curved rib on each side, springing from the anterior end of the centrum. The next two vertebræ doubtless have similar, but short ribs, though only the

proximal ends of them are seen. I do not think that the following vertebræ bore ribs, or, if so, they were much shorter. I can find no indications of such. In the restoration, these caudal ribs are shown directed backward, as in the matrix. Doubtless in life they were directed more downward, indicating a thick basal portion of the tail, probably compressed from side to side. Beyond the eighth visible caudal there is a break in the series, the only indication of contemporary extraneous force shown in the entire skeleton; and this may have been due to the fact that this part lies somewhat under the right hind leg in the nodule — that is, if the skeleton was originally fossilized in a prone and not supine condition. Following this gap there are three articulated vertebræ in line with the curvature of the basal portion of the tail. They are more slender than the preceding ones and clearly lie in their original position, the intervening five vertebræ having been dislodged, fragments of which are still preserved near the break. The extreme tip of the tail came near the margin of the nodule, at or below the tip of the muzzle, and has been destroyed by the erosion of the nodule. Perhaps a half dozen very small vertebræ are missing here, making altogether about twenty-five vertebræ for the number in the tail, or for the entire column about fifty, with a possible error of three or four more.

The dorsal ribs have an expanded proximal end, but without distinct differentiation into head and tubercle. The first one visible is attached to the vertebra concealed in part by the caudal end of the interclavicle, that is, the seventh or eighth of the series. It is altogether probable that the first four or five ribs were shorter, with expanded distal ends, as in *Labidosaurus*. From the eighth to the seventeenth the ribs lie nearly parallel on the right side, on the left being crowded more together at their ends. The longest of them measure forty-six millimeters along their considerable curvature. They are slender throughout. The broken ends of the eighteenth and nineteenth are visible in the matrix, but little if any shorter than the preceding ones. A fragment of what should be the twenty-first or twenty-second is also visible on the right side, but its length is not determinable. I have, therefore, shaded the last four ribs in the restoration, and it is possible these vertebræ were entirely ribless, as I have decided they were in *Labidosaurus*.

Pectoral Girdle. — The pectoral girdle lies almost perfectly in position, the hind end of the interclavicle only, turned slightly to the right, and the right coraco-scapula pushed slightly forward, or the left one backward. It is very certain that the girdle was attached immediately back of the skull, the front part underlying the occipital condyle even. In structure it is almost identical with that of *Labidosaurus*, as figured by me. The position in which they lie has slightly separated the clavicles at their scapula, attachment. I find no indications of a cleithrum. It is very evident that the coracoids in life were in immediate contact along the median line, covered over by the prolongation of the interclavicle. The scapulæ curve upward at an angle of about forty-five degrees from the plane of the coracoids. Possibly this angle has been reduced slightly by pressure, but I think not. The scapulæ are directed, not backward, as has been supposed, but obliquely upward.

Front Legs. — The humerus is of the usual shape, expanded proximally and distally in planes meeting each other at an angle of sixty or seventy degrees. The bone, like all other parts of the appendicular skeleton, is distinctly more slender than in *Labidosaurus*. The radius is a rather slender bone, cylindrical at its proximal, flattened and expanded at its distal extremity. The ulna, much broader and thicker at its proximal end, has a distinctly produced olecranon, and the curvature of the rather slender shaft in the middle is distinctly away from the radius. The most of the forearm and foot of the right extremity are preserved in the thinner block, the proximal ends of the radius and ulna in the thicker close by the right pubis. The hand, as thrust forward below the right mandible, is outspread and fully articulated, the middle of the wrist somewhat depressed by the angle of the mandible in the mud, slightly turning the distal end of the ulna. The terminal phalanges were probably present or but slightly dislodged, but their minute size has made it almost impossible to detect and work them out from the hard matrix. The carpus clearly agrees in its chief features with the carpus of *Labidosaurus* "*incisivus*," as figured by Case and myself, save that the parts were reversed in the figures. I give herewith a better figure of the labidosaur carpus, which has been more completely removed from

its matrix (Fig. 4). Because of the compression of the middle part of the carpus in our specimen, it is impossible to be quite sure of the presence of both centralia. The radiale is much broader than long, articulating with the radius, the distalia of the second and third digits, and with one or possibly two centralia. The ulnare is a much larger bone, articulating proximally with the ulna, distally with the two inner distalia, and on the outer side with the

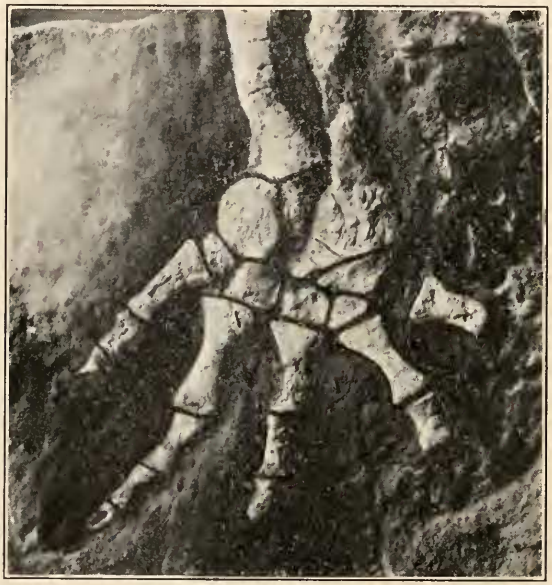

FIG. 3. *Pariotichus laticeps*. Photograph of right front foot, from thinner half of nodule; natural size.

intermedium. That there is a free intermedium here as in *Labidosaurus* is certain, but I cannot be quite sure of its extent, a part of it being apparently covered over by the radius. It articulates, as in *Labidosaurus*, proximally with the ulna and distally with a centrale. Five digits are present, as was to be expected. The first is represented by its metacarpal only, either slightly removed from its articulation with the radiale, or, what is more probable, with its distale lost, or cartilaginous. In the restoration it is shown removed from the carpal bones as in the photograph of the hand also given herewith (Fig. 3). The first metacarpal is the shortest of the five, and is only moderately ex-

panded distally. No phalanges are preserved. The second metacarpal is much longer than the first, and is much constricted in its middle. It has one short phalanx articulated with it, but little more than half the length of the metacarpal. Additional phalanges are not preserved, but, from its size, it seems very probable that two more, and not more than two, were originally present. The third metacarpal is much like the second, but is a little longer. Two phalanges are present, the first about two thirds the length of the metacarpal; the second fragmentary. There may have been a third, ungual phalanx present. The fourth metacarpal is the longest and stoutest of all, its proximal articulation more oblique than is the case with the preceding one. The first phalanx is about three fifths the length of the metacarpal. The second phalanx, much shorter and smaller, has at its tip a small fragment. There may have been a fourth phalanx, though there is not much probability of it. The fifth metacarpal is a little shorter than the fourth, somewhat curved and more slender. It has a small and short proximal phalanx and a fragment of a distal one at its tip. In all probability there were no more. It is, it is seen, impossible to say with certainty what the phalangeal formula of *Pariotichus* was, save that quite surely it was not that of the modern lizards and *Sphenodon*, 2, 3, 4, 5, 3. In much probability it was 2, 3, 3, 4(3), 2.

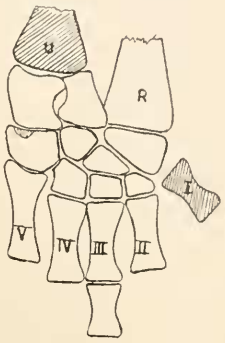

FIG. 4. *Labidosaurus*. Carpus; one half natural size.

Pelvis. — The under side of the pelvis lies in perfect preservation and position. It is, like the pectoral girdle, of the "old fashioned" type, elongate and plate-like, without thyroid foramen. The two sides meet in a long median symphysis, closely applied, but not sutural. The pubes, broadest in front, have a slight emargination in the middle in front. The small pubic foramen lies near the acetabulum, at the junction of the first and second thirds of the combined bone. Just inside this foramen, and a little to the outer side of the middle of each pubis, a pronounced thickening or ridge runs forward to the anterior margin, forming a

shallow fossa on each side of it. The pubo-ischial suture is, apparently, a little back of the pubic foramen, running transversely across. It is not at all distinct, and I am not sure of it. Lying in the median emargination of the pubes there are three or four very slender ventral ribs, and by their side, a fragment of what appears to be a thin plate, which may have articulated with the thickening of the front margin before mentioned, a thickening characteristic of all the smaller cotylosaurs, apparently. The pelvis, so far as it can be seen, is almost identical with that of *Labidosaurus*, and other Permian cotylosaurs, forms of which will be figured later.

Legs. — As stated, the hind legs are both preserved, trailing backward from the acetabulum, both of them somewhat bent at the knee. The femur of the right side suffered somewhat in the fracture of the nodule, and is only partly preserved. That of the left side, so far as it can be seen partly embedded in the matrix, agrees quite with that of *Labidosaurus*, though more slender. The tibia, also shown best on the left side, offers nothing unusual. The fibula, hitherto unknown in the cotylosaurs, is strongly curved, with a considerable expansion at its lower extremity, and with a small, rounded upper end. The feet, unfortunately, have both been preserved lying more or less on their fibular side, and in consequence with the toe bones more or less concealed. However, it is quite certain that no force was brought to bear upon them to displace them, save that of their own weight. The lower leg of the right side and the tarsus are spread out flatly, but with the digits piled upon each other. A large, flat fibulare articulates with the fibula of the right side in position, closely articulating on the inner side with another large bone, evidently the united tibiale and intermedium. Four tarsal distalia are visible. The shapes of the bones distinguished agree in general so well with those of *Labidosaurus* as figured by me, that I have no hesitation in giving the others from the same genus, shaded in the figure. The tibiale, however, must have been shorter than in *Labidosaurus*. As regards the toes, all five metatarsals are visible on one or the other side, and many of the phalanges, save those of the fifth toe. In the figure given in the restoration, the unshaded phalanges of the other toes are given precisely in the

positions they occupy with regard to the tarsus, so that the length of the toes is quite certain. Those phalanges which cannot be extricated from the matrix are shaded. In all probability the phalangeal formula is like that of the front feet; certainly there cannot be a greater number.

Six genera of Permian reptiles, all from Texas, are referred by Cope to the family Pariotichidæ; and, notwithstanding the difference in the teeth, I am disposed to add the seventh genus, *Labidosaurus*, to the same family. They are defined by Cope as follows:[1]

1. Teeth on the maxillæ and mandibles in a single series.....*Labidosaurus* Cope.
   Teeth in more than a single series.....2.
2. External nostrils inferior; mouth posterior in position; mandible short and with a few acute teeth.....*Hypnopous* Cope.
   External nostrils lateral.....3.
3. Palatal and splenial teeth with compressed crowns.....4.
   Palatal and splenial teeth obtuse, forming a grinding pavement; median maxillary and anterior incisor teeth enlarged.....*Pantylus* Cope.
4. Teeth equal, acute.....*Isodectes* Cope.
   Teeth increasing gradually in length anteriorly.....*Captorhinus* Cope.
   Teeth enlarged in the middle of the maxillary and anterior part of the incisor series.....*Pariotichus* Cope.

The genus *Helodectes*, provisionally placed in the Pariotichidæ, is distinguishable by the two rows of teeth on the jaws, the "bases of which are wide ovals, transversely placed."

Isodectes is figured by Cope as having the prefrontals and postfrontals meeting broadly over the orbits, widely excluding the frontals from the orbit. The skull, moreover, is much longer than broad in the type species, *I. megalops*. *Pantylus* also has the prefrontals and postfrontals meeting broadly as in *Isodectes* in the type species, *P. cordatus*, which, moreover, has a rounded muzzle, and is widely expanded posteriorly. *Captorhinus* has an elongate, pointed skull, with the orbits twice the diameter of the interorbital space. *Hypnopous* is wholly out of consideration because of the remarkable position of the nares. Assuming that our species has more than one row of teeth on maxillæ and mandibles, its exclusion from the labidosaurs is of course evident. As that character cannot be determined save by the mutilation of the otherwise perfect skull, the doubt must be left. By ex-

[1] *Proc. Amer. Phil. Soc.*, XXXIV., 1895, p. 445.

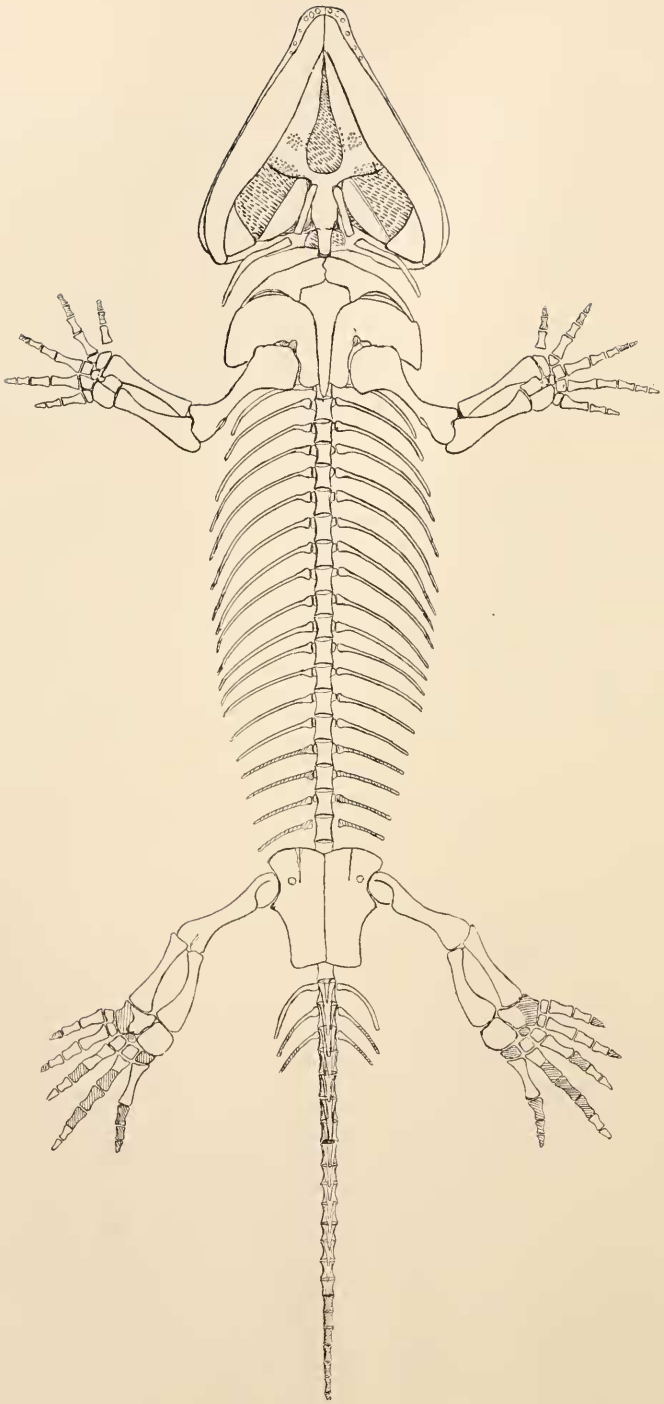

FIG. 5. *Pariotichus laticeps.* Restoration of skeleton ; one half natural size.

clusion, we have only the genus *Pariotichus* left, and in all its characters, so far as they have been developed in the known species of the genus, the agreement seems sufficiently certain. Six species of *Pariotichus* have been described. Of these, *P. brachyops* is excluded by the large maxillary tooth not being below the anterior border of the orbits, but much further forward, by the relative size of the orbits, etc. *P. aguti* is easily distinguished by the elongate shape of the head, its less depressed form, etc. *P. isolomus* differs distinctly in its less expansion posteriorly, its length being distinctly greater than its width, and the absence of a posterior emargination of the cranial border. *P. incisivus* has been wrongly identified as having a single row of teeth on mandibles and maxillæ by both Case and myself, whereas Cope distinctly figures it (*Trans. Amer. Phil. Soc.*, 1886, p. 290,

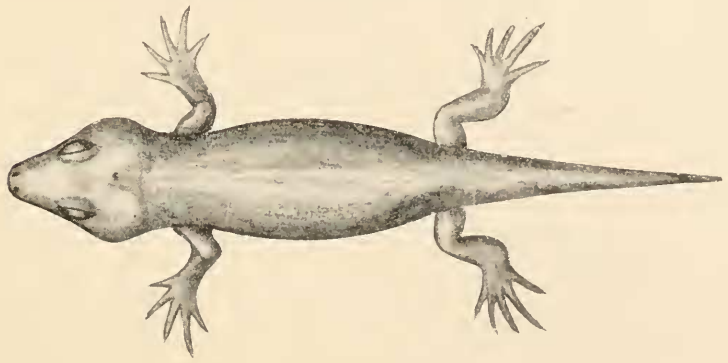

FIG. 6. Life restoration of *Pariotichus laticeps*; one fourth natural size.

Pl. II., Figs. 4 and 5) as having additional teeth. The form described by me as *Labidosaurus incisivus* is therefore something else. *P. incisivus* is described and figured by Cope as having a purely reticulated sculpture of the skull, which he accepts as of specific value. Our species has the sculpture, distinctly longitudinal, on the upper side of the skull anteriorly at least. *P. aduncus* is distinguished by the size of the orbits, etc. We have, then, but a single species left, *P. ordinatus* Cope, described from such scanty material that it is doubtful if an actual comparison of the type specimen would resolve doubt as to its identity with the species herein described. Our species may, therefore, be provisionally given the name of *P. laticeps*.

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BIOLOGICAL BULLETIN

SPERM-TRANSFER ORGANS IN CAMBAROIDES.

E. A. ANDREWS.

Amongst the arthropods it is not uncommon for some of the limbs to be used for transferring the sperm from the male to the female. In some of these cases the path of the sperm from the male to the egg is very complex, and we may speak of "indirect sperm-transfer."

Thus in the common crayfishes of the United States, of the genus *Cambarus*, the first and the second limbs of the abdomen of the male are special organs that we will call the first and the second stylets, which conduct the sperm from the male openings upon the bases of the fifth pair of walking legs to the surface of the female. In the female the sperm is received into a pouch in the shell on the under side, between the fourth pair of walking legs. This sperm receptacle is hollowed out in the so-called annulus, or special sternal plate, and in this pouch the sperm remains till the eggs are laid. In the crayfish of Europe, however, the sperm conveyed by the stylets is deposited freely over the sternum, in secreted tubules, or spermatophores, and there is no sperm receptacle.

There are then two forms of indirect sperm transfer within this family, in the two genera *Cambarus* and *Astacus*.

The crayfish of Japan and the Amoor River region are so different from other species of *Astacus* that it is a question whether they do not form a distinct genus. They resemble *Cambarus* so much that Faxon called them *Cambaroides*. Nothing is known as to the method of sperm transfer in this *Cambaroides* subgroup of *Astacus*, but the following account of the anatomy of the organs concerned may aid in a tentative view as to what actual observation of the process may reveal.

The material used was kindly loaned by the National Museum,

and consisted of a very few specimens of *Astacus* (*Cambaroides*) *similis* from Corea and some ten specimens of *Astacus* (*Cambaroides*) *Japonicus* from Hakodate, Japan. These last were obtained by the "Albatross" from the market in July, 1906, and were remarkable in being all strung along upon bits of stick that had been thrust through several crayfish, one after the other, passing through the head-thorax and abdomen lengthwise. From the condition of internal anatomy the specimens would appear to have been dried before they were preserved by the naturalist.

Observations upon *Cambarus* have shown that when the stylets are being used to fill the sperm receptacle the male is firmly fastened to the female by two pairs of hooks, or spines, that stand out like spurs from the walking legs and are carefully fitted into the groove between the segments of the legs of the female. In fact, experiments show that without these spines the

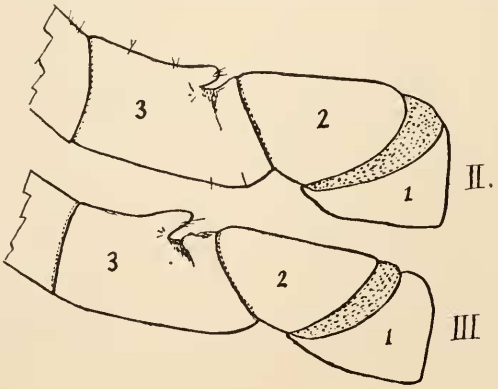

FIG. 1.

other organs are of no avail as the male is not able to transfer the sperm to the sperm pouch. In *Cambarus* there are then three sets of necessary external organs concerned in sperm transfer, the hooks and the stylets of the males and the sperm receptacles of the females.

In *Cambaroides* the hooks are present as well as the stylets but in the females there is no discovered receptacle though the annular plate is somewhat modified.

We will describe these three sets of organs, in the following order: hooks, stylets, annular plate of female.

In *Cambaroides similis*, a specimen 55 mm. long had the hooks developed as in Fig. 1, a blunt rounded spine upon the third segment of the second and the third walking legs. Each spine

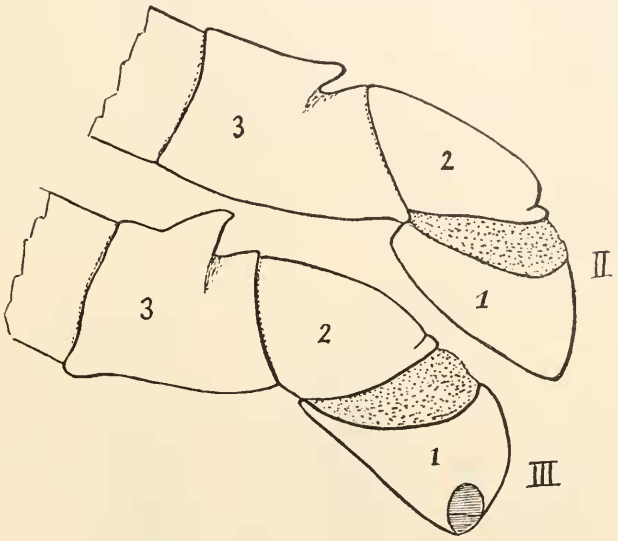

FIG. 2.

bears a few setæ. They are like the spines of *Cambarus Montezumæ* in being on the second and third legs, but are much shorter, more blunt and less effective as hooks. The spine of the third leg is the better developed.

In *Cambaroides Japonicus*, Fig. 2, the hooks are essentially the same, but more pointed. In both cases the resemblance of the hooks to those used by *Cambarus* is so strong that one would infer that they are probably functional in *Cambaroides*.

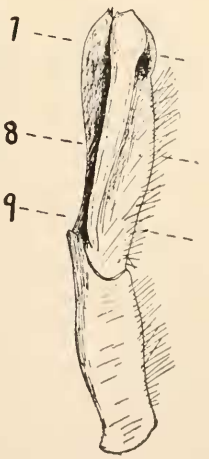

FIG 3.

In comparing the stylets of *Cambaroides* with those of *Cambarus* we note that the first lacks the fine detail of apex commonly found in *Cambarus* and is a more stout and undifferentiated organ. In *Cambaroides similis*, Fig. 3, the first stylet is a clumsy cylinder having a movable joint between the long protopodite and the somewhat longer distal endopodite.

Both bear a fringe of setæ along the external edge. This long line of setæ seems to represent what is found upon the edges of the common pleopods and may be regarded as a mark of little specialization or of retention of primitive characters, since in *Cambarus* it is generally specialized as a local group of setæ, or at most in *C. Clarkii*, as a less simple line.

The main features of the endopodite are, however, a shallow groove and a very stout ridge along the posterior face, external to the groove. This ridge ends distally as a swelling that is part of the specialized tip of the organ. More enlarged, Fig. 4,

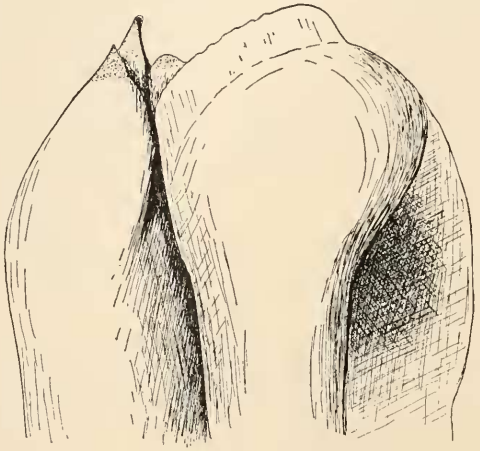

FIG. 4.

the tip shows the rounded head in which the ridge ends, the groove to the left of it and a depression to the right. The actual ending of the organ is a sort of complex edge, flattened from before back.

On the median side of the groove are two horny points, the shorter posterior, the longer anterior. The posterior is comparable to the large "spatula" and the anterior to the large "canula" of *Cambarus Montezumæ*. External to the groove is a short, blunt anterior point, comparable to the "ligula" of *C. Montezumæ* and a long thin knife edge that is a continuation of the rounded head of the ridge. The organ appears fitted to open a slit into which sperm might flow from the groove.

In *C. Japonicus* the two first stylets lie side by side with diver-

gent tips. In a male 45 mm. long, with stylets 8 mm. long, their tips were two millimeters apart, across the median line. Each stylet has the same structure as in *C. similis*, with only slight differences in proportion, but the groove opens more toward the median face and is not seen from behind, Fig. 5. While the tip Fig. 6 presents the same details as in *C. similis*, Fig. 4, the cutting edge is less sharply set off from the head of

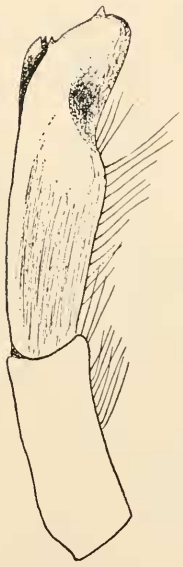

FIG. 5.

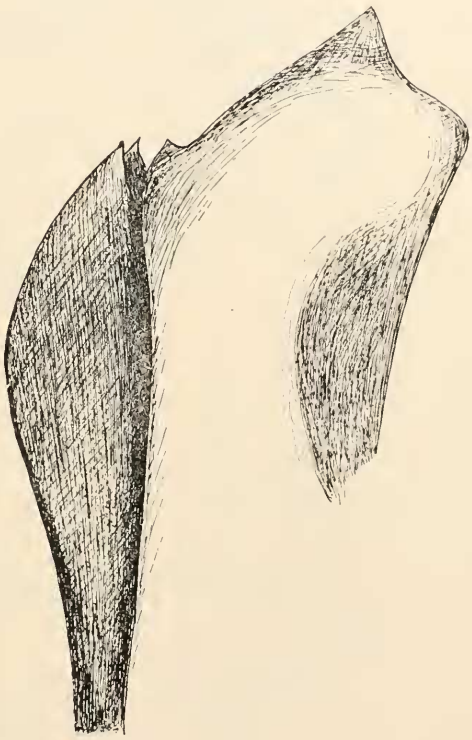

FIG. 6.

the ridge and runs out externally as a pronounced angle or spine, that is lacking in the former species.

From an end view the tip of the organ of *C. Japonicus* is complexly modelled and suggest not a cutting tool but a probe to be forcefully inserted against resistance.

Serial sections of the first stylets of both species show the same inside structure. A thick, firm shell covers the soft

areolar tissue and a delicate epidermis underlies the shell and is continuous with the areolar tissue. The internal anatomy and the external modelling is shown by the series of sections, Figs. 7, 8, 9, cut along the lines 7, 8, 9 of Fig. 3. The organ is essentially a thick, flat plate with a groove on its posterior face dividing it into a smaller median part that we will call the median mass and a larger external mass, *M.M.* and *Ex.m.* in Fig. 7.

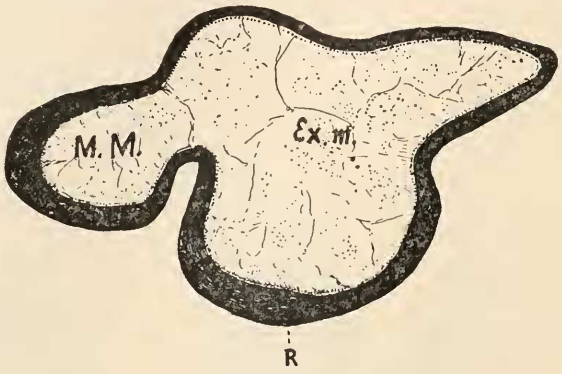

FIG. 7.

The groove is made much deeper by the fact that a great ridge, *R*, rises up from the external mass and forms the external boundary of the groove. In the middle of the course of the groove the ridge, Fig. 8, extends toward the middle line of the

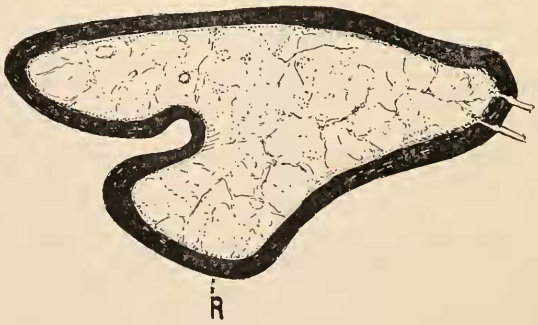

FIG. 8.

body parallel to the median mass so that the groove is here deep and narrow and opens out more to the median face of the organ. Toward the basal end, Fig. 9, the shallow groove is bounded by

the diminishing terminal part of the ridge. At this level also may be seen the muscle mass that extends into the endopodite and indicates that the joint between the endopodite and exopodite may actually be used and the position of the tip of the organ be

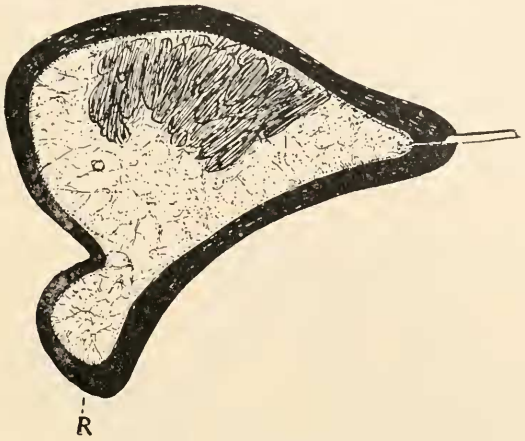

FIG. 9.

directed by this muscle. This muscle was also seen in cleared mounts in toto.

In the same manner in *C. Japonicus* sections show only a simple groove and large ridge, with the only difference that the groove faces more toward the median aspect of the organ, so that the above Figs. 7, 8 and 9 would well represent the condition in both species.

The second stylet preserves the usual pleopod form in that it is forked, or has both endopodite and exopodite. In *Astacus* (*Cambaroides*) *Japonicus*, Fig. 10, the exopodite is a slender obscurely segmented filament bearing few long setæ while the endopodite is the wide massive terminal part of the stylet. The tip of this endopodite, the flabellum, bears a long tuft of setæ and is evidently like the tip of the exopodite, but much enlarged. In the median edge of the endopodite there arises the extra element comparable to the "triangle" of *Cambarus*, that probably has some use in sperm transfer. This is a thick ridge that rises up as a free, thumb-like process directed diagonally across the endopodite. It has a marked angular elbow on the median side

and terminates in an oblique and somewhat hollowed face posterior to the flabellum. This very simple representative of the triangle of *Cambarus* and the scroll of the American and European *Astacus*, bears still a few setæ, several upon the median and two or three upon the external border. In this it is intermediate between the above two crayfish.

The entire appendage seems crude and clumsy, either primitive or reduced.

This appears again in the other species of *Cambaroides*, *C.*

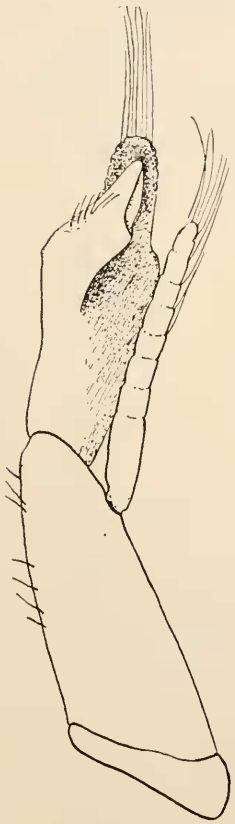

Fig. 10.

Fig. 11.

similis, Fig. 11, which is like *Japonicus* but the setæ are very short and the flabellum more reduced. The dotted area in Fig. 11 is membranous. The posterior face is turned so that the

triangle can fit into the groove in the first stylet. In *Cambarus* the projection of the second stylet fits accurately to the groove of the first stylet and insures sperm transfer and in *Cambaroides* we can see that the projection upon the second will run in the groove of the first but it does not seem nicely adjusted to it.

Another departure from the finer adjustments of *Cambarus* may be inferred from the simpler mode of ending of the defferent duct. While in *Cambarus* it ends in a soft papilla that is fitted

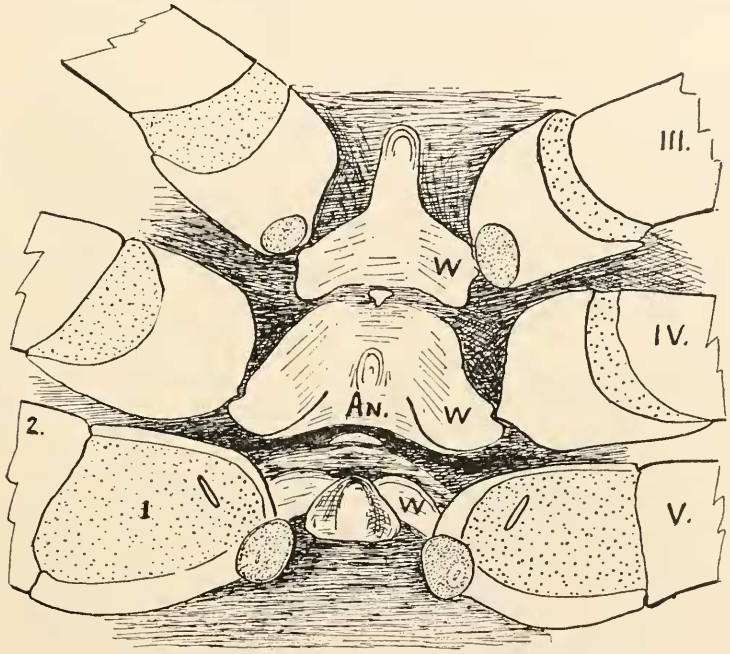

FIG. 12.

into the groove of the first stylet, in *Cambaroides* there is, at least in the preserved specimens, only a rounded, slightly raised area with a slit in it for the exit of the sperm.

The under side of the thorax of the male, Fig. 12, shows the ending of the defferent duct as a small opening in a rounded raised area on the base of the fifth leg, right and left. In *Cambaroides Japonicus*, Fig. 12, these rounded areas are soft and the entire adjacent surface of the base of the leg is also membranous, as indicated by the dotted region, except for the minute hard

oblique ridge isolated in the membranous area. In *Cambaroides similis*, the base of the fifth leg shows a large white area thought to be glandular, the area 1 in Fig. 13, and the ending of the defferent duct is in a solid projection with a slit-like orifice.

Whether in life a soft papilla can be projected from these

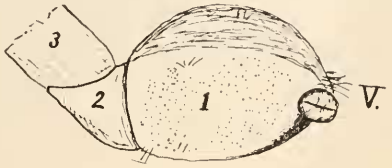

Fig. 13.

orifices is doubtful, but the hard and slightly projecting areas can be put against the groove in the first stylet in such a way that we infer the sperm may be poured out into the groove of the stylet, with perhaps some aid from the second stylet.

Turning now to the destination of the sperm transferred by the

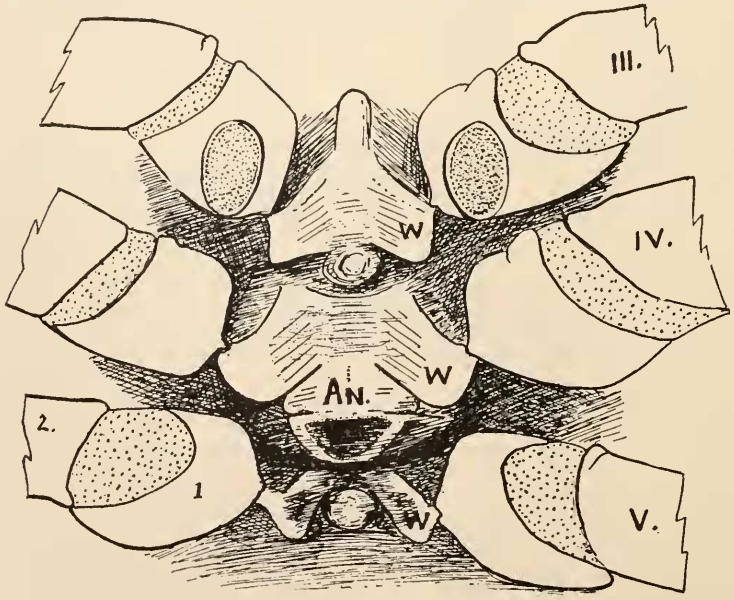

Fig. 14.

above male organs we fail to find upon the female any specialized receptacle. The under side of the thorax of the female *Cambaroides Japonicus*, Fig. 14, has a series of median plates between

the limbs *III*, *IV* and *V*. Each plate expands toward the limbs as a lateral wing, *W*, each plate is also modified in its central part. Between the third limbs the central part shows a rounded boss and an anterior prolongation ; between the fifth limbs there is only a boss ; between the fourth limbs the median part of the plate is prolonged backward not as a boss but as a large expanse, the annular plate, *An*. This annular plate is subdivided into an anterior part, *An*, somewhat convex from side to side and a posterior part which is hollowed out as is poorly shown in Fig. 14.

The hollowed posterior part of the annular plate rises up dorsally at an angle with the horizontal anterior part.

The median depression is quite shallow and neither in surface views nor in sections is there any slit or internal pocket such as is characteristic of the annular plate of the genus *Cambarus*. Thus while in both *Cambarus* and *Cambaroides* there is an annular plate only in *Cambarus* is it provided with an internal cavity. It is the internal cavity in the annular plate that is filled with sperm. In these specimens of *Cambaroides* no sperm pocket is found.

In the male *Cambaroides*, Fig. 12, there is also an annular plate between the fourth limbs but it lacks the hollowed posterior part that is found in the female.[1]

In a specimen of *Cambaroides similis* the posterior part of the annular plate is less sharply hollowed out than in *C. Japonicus*.

The use of these various male and female parts can, as yet, only be inferred from comparisons with the organs of *Astacus* and of *Cambarus* whose use has been observed. But in applying the male to the female we are led to imagine that the stylets may deposit spermatophores between the fourth and fifth limbs.

[1] In the individual male figured here there is an abnormal pair of structures that simulate the openings of oviducts. In the female the oviduct openings, Fig. 14, are large elliptical membranous areas upon the bases of the third limbs. In the male the openings of the defferent ducts are more elevated areas upon the bases of the fifth limbs, Fig. 13. There is thus both a difference in position and in character between the male and the female openings. The abnormal openings upon the third legs of the male figured in 12, are like female openings both in position and character though they are smaller than normal and the one upon the right of the animal is especially small (see also Fig. 2). This then seems to be a case of partial mixture of sex organs such as have been described in both *Astacus* and *Cambarus* (see *Am. Nat.*, 1909).

On the whole the sex organs of *Cambaroides* are more like those of *Cambarus* than like those of *Astacus*. That is, the hooks are present in *Cambarus* and *Cambaroides*, but not in *Astacus*. From their form we may infer that in *Cambaroides* they are used to hold the female just as they are used in *Cambarus*. The stylets in *Cambaroides* lack the flat scroll form of *Astacus* and are more like the stout complexly tipped organs of *Cambarus*, but they are much more simple. They doubtless serve to transfer the sperm as in both *Astacus* and *Cambarus*. In the female the annular plate of *Cambaroides* lacks the special sperm reservoir of *Cambarus* and is thus like *Astacus*, but it is more developed and somewhat hollowed out. In this respect it recalls the earliest phase of the ontogeny of the annulus of *Cambarus*.[1]

In brief the organs of *Cambaroides* are more simple than those of *Cambarus* but fashioned somewhat like them, suggesting some connection closer with *Cambarus* than with *Astacus*.

How are these facts to be interpreted? The general anatomy shows that *Cambarus* is the recent and *Astacus* the more unspecialized genus. Is *Cambaroides* a step from *Astacus* toward *Cambarus* or is it a step backward from *Cambarus?*

Since *Cambaroides* has the same gill formula as *Astacus* and is like *Astacus* in having no sperm receptacle (as far as known), while on the other hand it has hooks like *Cambarus* and stylets similar to those of *Cambarus*, we may regard it as a genus separate from both *Astacus* and *Cambarus*.

It then becomes a question of the relative positions of these three genera. Granting that the larger number of gills is primitive and the small number derived we must assume either that the presence of hooks in *Cambarus* with few gills and in *Cambaroides* with more gills is a case of secondary convergence from parallel variation or else that it is a common inheritance. Ortmann has assumed the resemblances of *Cambarus* and *Cambaroides* due to convergence, but Faxon regarded them of more significance. The new facts as to the annular plate and the structure of the stylets will aid in the solution of this question; with emphasis upon the sex organs as criteria of relationship, which has been the tendency of all recent work upon this group.

[1] Biol. Bull., X., Figs. 6, 7.

As elsewhere shown[1] some of the Penæidæ as well as the lobsters have sperm receptacles whence we may infer that a sperm receptacle was common to the ancestors of the crayfish. In such case the absence of sperm receptacle in *Astacus* would be due to loss, and the presence in *Cambarus* to retention of the ancestral mode of sperm transfer. If we suppose that the resemblances and differences of organisms are connected with chance variations it seems more likely that organs may have been independently lost, in separate animals, than that the same organ should have been independently acquired in separate organisms. We would then say that *Cambaroides* and *Cambarus* are closely related as both have retained the hooks and the general form of stylets of some ancestor but that *Astacus* and *Cambaroides* are not so closely related though both have independently lost the sperm receptacle while changing the form of stylets.

Adopting Ortmann's views as to the origin of the present distribution of crayfish we would modify it chiefly by the assumption of two migrations from Asia into America. We would think of ancestral Asiatic crayfish with many gills and a sperm receptacle of some sort filled by some use of the abdominal limbs. One set of descendants retaining more gills but losing the sperm receptacle became the *Astacus* of America and Europe as well as the crayfish of the southern hemisphere. While another set of descendants became *Cambarus* and *Cambaroides*. Part of this branch migrated into America and ultimately, in Mexico according to Ortmann's evidence, became the present *Cambarus* with reduced gills and highly specialized receptacle and stylets. The other part remaining in Asia independently lost the receptacle but retained the larger number of gills as well as the hooks.

We might then find in *Cambaroides* indications of the former presence of a sperm receptacle. As such we regard the hooks that have no known use except as aids for the filling of a receptacle. As such we regard the presence of ligula, spatula and canula at the tip of the stylet.

The absence of a tubule in the stylet, its clumsy form and the reduced prominence of its tip as well as the simplicity of the triangle of the second stylet might also be regarded as signs of degeneration.

But speculation is here very insecure and if use inheritance or some law of perfection were known it would be easy to argue that *Cambaroides* was an incipient *Cambarus* evolving from *Astacus*. In any case the common ancestor of *Cambarus* and *Cambaroides* must have been far back as it had the larger number of gills and as *Cambaroides* has the primitive characters of a well-developed flagellum on the first stylet and a muscle at the movable joint between protopodite and endopodite.

A diagram of the three genera would place *Astacus* and *Cambaroides* near together as having the same gill formula and as lacking a sperm receptacle, while *Cambarus* should stand apart as having a simplified gill formula and also very highly developed sperm-transfer organs, including a sperm receptacle. At the same time the diagram should indicate that *Cambarus* and *Cambaroides* kept together after departing from *Astacus*, and that later *Cambaroides* went off in the direction of *Astacus*, leaving *Cambarus* as at once the most specialized in its gills and the most conservative in its retention of the very ancient crustacean mode of sperm transfer by the employment of a sperm receptacle.

BALTIMORE, June 10, 1909.

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN THE DOMESTIC FOWL.

III. A Case of Incomplete Hermaphroditism.[1]

RAYMOND PEARL AND MAYNIE R. CURTIS

Origin and General Character of Specimen.

From a chick hatched in the spring of 1907, at the Maine Agricultural Experiment Station, there developed the bird which forms the subject of this paper. This bird was a Barred Plymouth Rock and when adult presented externally the general appearance of a normal hen of this variety, so far as the characters body form and plumage color were concerned (cf. Plate I.). As the photograph in Plate I. shows, however, the head and neck resembled these parts in a cockerel. This resemblance was especially remarkable in respect to the size and shape of the comb and wattles. The comb was obviously much larger than the comb of a normal Barred Plymouth Rock hen and looked exactly like the comb of a male bird. This was also true of the wattles.

The dimensions[2] of the comb of this bird were as follows :

Length	88.4 mm.
Calculated height	25.1 mm.
Area	22.2 cm.^{2}

For normal adult Barred Plymouth Rock females the following average values for comb size have been found :[3]

Mean length	50.80 $\pm$ .56 mm.
" calculated height	10.57 $\pm$ .23 "
" area	5.59 $\pm$ .17 cm.^{2}

It is evident from these figures that the comb in this specimen greatly exceeds in size the average for females of the variety.

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 13.

[2] Made in accordance with the methods described by R. and M. D. Pearl in a paper "Data on Variation in the Comb of the Domestic Fowl," *Biometrika*, Vol. VI., pp. 421–423.

[3] Pearl, R. and M. D., *loc. cit.*, p. 427.

In regard to behavior this bird resembled a normal hen rather more than a cock. She was never heard to cluck, however, or to make any of the sounds which normal active hens make in the course of the day's work. This bird probably never laid an egg, though we are unfortunately not able to make an absolute statement on this point. The egg records of the station show an egg to the credit of this bird on November 7, 1907. This was the only egg ever recorded for this bird, and it is undoubtedly an erroneous record. As will presently appear, the condition of the sexual organs was not such as to indicate that they had ever been functional.

Cockerels placed in the pen with this bird would try to fight with her as if she were a cockerel; but she would not fight.

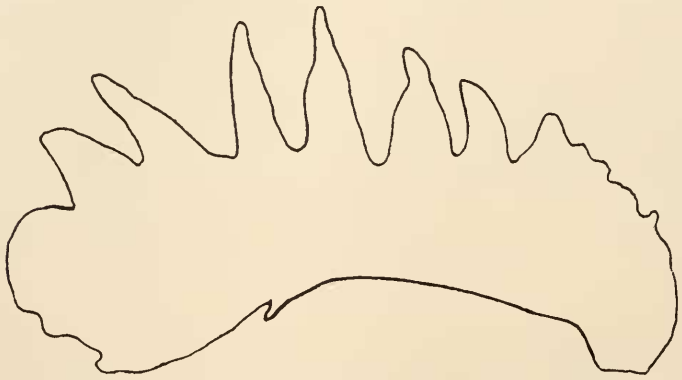

FIG. 1. Outline of the lateral aspect of the comb of the Barred Plymouth Rock hen described in this paper. This outline is actual size.

We have no evidence that a cockerel ever attempted copulation with this bird. These facts are of interest in relation to the question of the basis of sex-recognition and the assortative mating known to occur among fowls. Is a normal pullet with an unusually large comb less likely to have her eggs fertilized than a bird with a smaller comb?

This bird was observed occasionally to take the position of a cockerel about to crow and attempted to crow but never succeeded in very closely approximating the sound of a normal cock bird. The bird was never seen to attempt to tread a hen.

Autopsy.

The appearance and behavior of this bird led to the suspicion that it represented a case of true hermaphroditism. On August 24, 1908, the bird was killed and a post mortem examination made. The weight of the body after bleeding was 2,725 grams. The body cavity contained much fat. The alimentary tract and attached viscera were entirely normal. The following measurements were made:

From gizzard to origin of cœca	167 cm.
Longest cœcum	22 "
From origin of cœca to cloaca	13 "

The following weights were taken:

Gizzard	125 gms.
Liver	44 "
Heart	9.5 "
Spleen	4.5 "

On the left side of the body was a normal oviduct. There was an ovary in the usual position. It was of about the size of the ovary of a laying hen after the removal of large yolks. It had a coarsely granular appearance and showed many folds. There were no eggs visible and its surface did not have the ragged appearance, due to ruptured follicles, which is characteristic of the ovaries of laying hens. The rest of the urinogenital system was completely covered by fat. The part of the body containing this organ system was hardened in formalin for further dissection.

Gross Anatomy of Reproductive Organs.

Dissection confirmed the suspicion of hermaphroditism. On the left side were the female-like reproductive organs described above while on the right side there was a set of organs similar to those of the normal male. The gross anatomy of the reproductive system in this bird is shown in Plate II., Fig. 1.

The female organs were more nearly normal than the male. The ovary, like a normal ovary, was ventral to the craniad lobe of the left kidney, covering, when viewed from the ventral side, all but the caudo-lateral angle of this lobe. It extended past the cranial margin of the kidney in the hermaphrodite nearly to, and in the normal hen with which comparisons were made, slightly beyond the fourth rib. In the hermaphrodite the ovary

was developed less on the medial side than in the normal hen. The measurements of greatest length and breadth of ovary in the hermaphrodite were 29 mm. by 16 mm. while in the normal hen they were, excluding the projecting yolks, 34 mm. by 20 mm. The ovary was attached to the body wall near the middle line by a thick stalk-like portion. This appeared perfectly normal. Its longest dimension (cranio-caudal) was 15 mm. compared to 18 mm. in the normal bird. The external appearance of the ovary was quite different from that of a normal ovary. It seemed to be a coarsely granular but otherwise homogeneous mass covered by peritoneum and minutely and very irregularly folded. It did not have the ragged appearance of the normal ovary and the minute folded masses did not look like the yolks of similar size in the normal ovary. They seemed to be folds on the surface of a homogeneous mass rather than small spheres of yolk enclosed in follicles.

The oviduct was normal in appearance and in position. The mouth of the funnel faced the ovary while the cranial ends of the lips were fused and extended across the left kidney to the fourth thoracic rib some distance laterad of the cranial end of the ovary. The caudal ends of the lips also fused and were attached to the ventio-median margin of the ligament which holds the convolutions of the oviduct in place. The oviduct presented the same principal convolutions as a normal active oviduct. It was larger than the oviduct of the adult hens we have examined which had never laid and the ovaries of which did not show a number of yolks. Table I. gives the lengths of the oviducts we have been able to examine in this condition.

TABLE I.

Dimensions of Oviducts of Pullets which have Never Laid and which have no Growing Yolks on Ovary. Hermaphrodite Included for Comparison.

Band Number of Bird.	Length of Oviduct in cm.	No. of Yolks Above 1 cm. on Ovary.	Total Number of Eggs Laid.
Hermaphrodite			
16 D	47.0	0	0
129 E	13.0	0	0
297 E	20.0	0	0
222 E	23.5	0	0

The length of the oviduct of the hermaphrodite hen 16 as

given in the first line of this table is 47.0 cm. This is just twice the length of the next shorter oviduct, that of hen 222 E. The oviduct is however smaller than in hens actively engaged in egg production in the middle of a laying period. It compares most nearly in size with the oviduct in hens which have ceased laying from 5 to 16 days before examination, or to those with 4 to 6 large yolks on the ovary. In order that this comparison may be readily made Table II. is introduced. This table is compiled from records in the archives of the laboratory and gives certain data in regard to normal hens having oviducts between 44.5 cm. and 50.5 cm. long, *i. e.*, of approximately the same length as that of the hermaphrodite hen No. 16. The table includes the following data: (*a*) length of oviduct in cm.; (*b*) the number of yolks 1 cm. or more in diameter on the ovary at the time of autopsy; (*c*) the number of days elapsed since the last egg was laid. In case the bird never laid the sign ∞ is used to denote this fact.

TABLE II.

Data from Normal Hens on Oviducts of Same Size as that of Hermaphrodite Bird.

Band Number of Bird.	Laboratory Autopsy Number.	Length of Oviduct in cm.	Number of Eggs 1 cm. or More in Diameter on Ovary.	Number of Days Since Last Egg Was Laid
Hermaphrodite 16	175	47.0	0	∞ (?)
260 D	117	47.0	0	8
15 D	124	50.0	0	5
213 D	133	46.0	0	7
788 D	137	45.0	0	9
38 D	151	46.0	0	8
405 D	171	50.0	4	9
317 E	179	50.0	4	∞
480 E	202	49.0	2 absorbing	16
314 E	236	48.5	2 "	10
96 E	237	49.0	6 "	∞
429 E	248	48.5	14 (hard, absorbing)	9

From the table it is apparent that the oviduct in the hermaphrodite hen was in essentially the same condition as that of a normal hen which has recently completed an egg-laying period (clutch).

Internally the oviduct of the hermaphrodite was differentiated into the regions characteristic of the normal oviduct. The funnel walls were thin and transparent. Grandular ridges appeared

at the end of the funnel and became gradually heavier and higher. In the albumen-secreting portion of the tube they were heavy, high and irregularly lobed. The ridges at the cranial end of the isthmus were thin and straight but did not preserve that character so strictly as in a normal oviduct, so that near the shell gland they resembled the ridges in the albumen-secreting portion. The shell gland ridges were high, very irregular and much lobed. These turned dark in the preserving fluid as we have often noticed to be the case with normal oviducts. The vagina had the characteristic low straight ridges. The dimensions of the various parts of the oviduct were as follows :

Length of funnel neck	2.5 cm.
Width of flattened tube at point where funnel passes into albumen portion	0.6 "
Length of albumen portion	18.5 "
Width of flattened tube at widest part of albumen portion	0.9 "
Length of isthmus	7.0 "
Length of shell gland	7.5 "
Width of widest part of shell gland	2.0 "
Length of vagina	11.5 "

The opening of the oviduct was in the normal position, slightly to the left of the midventral line. The margin of the opening was folded, but was inconspicuous while in a laying hen it protrudes a little into the cloaca. A large probe was passed from the vagina into the cloaca demonstrating a natural opening between these organs.

The left suprarenal body was covered by the cranial end of the ovary as in normal cases.

Directly opposite the middle of the ovary on the left side of the body was a small irregular, though generally ovoid organ, the testis (Plate II., *T*). This organ was 9 mm. in length by 6 mm. in greatest breadth. It was attached to the body wall by its broad side with the more convex side median and the nearly straight side lateral. This organ did not appear macroscopically like a testis but looked to the naked eye or through the hand lens, like a small mass of the same sort of tissue as the ovary already described, but covered with an additional layer of connective tissue which obscured the minute foldings.

From the lateral side of the testis a duct passed to the cloaca

running parallel to the median line and ventral and lateral to the ureter. This tube was nearly straight throughout its course, but had a few convolutions near the cloacal end. It had the position and appearance of a normal vas deferens in a young cockerel. The tube was heavy walled and gradually increased in diameter caudad. Sections showed that this duct had a definite lumen. There was no enlargement comparable to a seminal vesicle. It was not possible to demonstrate an opening into the cloaca.

Histology of the Left Genital Gland (Ovary).

The left genital gland was much less finely lobulated than a normal ovary. The large lobules had smooth contours. The organ was covered with a layer of peritoneum. Over most of the surface the cells of this layer were nearly cubical but in some portions they were shorter than broad while in other regions they were nearly twice as tall as broad. Over a few small areas there was an outward proliferation of this epithelium so that evaginated folds of the epithelium four to six cells deep projected from the surface. In a few cases these evaginated ridges were still further folded along their lateral margins.

Beneath the peritoneal layer was the tissue which formed the bulk of the organ. This was a highly cellular but much vacuolated tissue, the cells of which were not unlike the cells of the stroma of a young ovary. This tissue was nearly uniform throughout the organ. In the vacuoles of this tissue were found, in many portions of the organ, irregular non-cellular masses which stained deeply with acid stains, especially eosin. Some of these masses were surrounded by a single layer of very much flattened cells. They did not appear like ova nor did the surrounding cells resemble normal follicle cells. In the part of the organ ventral to the suprarenal body were a few spherical portions of the stroma-like tissue which were more dense and took a deeper stain with hæmatoxylin. These portions did not differ in other particulars from the surrounding tissue.

The stroma-like tissue contained few blood vessels but a highly vascular connective tissue penetrated the organ from the stalk. This tissue appeared like a core to the organ projecting into the larger lobules in tongue-shaped masses.

No Graaffian follicles or Pflüger's tubes were found, though series of sections from all parts of the gonad were examined. The general histological structure of the organ was such as to indicate that it was in a degenerating condition at the time the bird was killed. This process of degeneration had gone so far that nothing like normal ovarian tissue was to be found. Whether at any time in the life of the bird any part or all of the ovary had been normal in structure, it is impossible now to say. The condition of the gland at death afforded no certain evidence either for or against this view. That oögenesis, however, could not have gone beyond early stages during the later life of the bird is made probable by the fact that it did not lay (except for the single doubtful egg noted on p. 272), although possessed of a normal oviduct.

The net result of the microscopical examination of the left genital gland, which had the normal anatomical relations of an ovary, is negative.

Histology of the Right Genital Gland (Testis).

The limiting membrane of the right genital gland was not very thick and was poorly preserved in our sections. Such parts of it as were intact seemed to have a cellular outer layer with a fibrous inner layer. We could not be sure of futher histological details nor could we determine the extent of this tunic.

The gland contained no normal seminiferous tubules but showed evidence of tubular origin. The central portion was more dense than the periphery and in this more dense portion a few places showed the cells arranged as if small cellular rods had been cut in various planes. These rods might be considered tubes without lumen. They were formed by a single layer of nearly cubical cells, about the size and form of the epithelial cells of the seminiferous tubules at the age when these form a single layer nearly filling the lumen. Around these rods was a thin layer of fibrous tissue. Between the dense central portion and the periphery the epithelial cells gradually disappeared so that the greater portion of the gland appeared to be a connective tissue skeleton representing the basal membranes of the tubes, and the intertubular stroma of the young gland. Most of the tubes formed by the remaining basal membranes contained a few

cells but these were irregular and had lost their epithelial character. In some of the tubes were eosin-staining non-cellular masses like those found in the left genital gland. In the right gland we did not find cells surrounding these masses.

On the dorsal side of the organ was a mass of tubules. Those seen in each section varied considerably in diameter but they were much smaller toward the cranial end of the organ. This mass of tubules extended the full length of the testis. The tubes were lined with simple, heavily ciliated, columnar epithelium. Outside this, especially in the larger tubules, could be distinguished one or more layers of smooth circular muscle cells. The tubes were imbedded in connective tissue. This tubular structure was in all essential particulars precisely like a normal epididymis and without any question represents that organ. A photograph of a section through the epididymis is shown in Plate II., Fig. 2. The magnification used in this figure is low, but on the original negative the cilia on the cells lining the tubes can be plainly seen.

On the median side of the testis and lying for the most part at the side of the epididymis, though in some portions extending between the gland and the epididymis, was a mass of very vascular connective tissue.

Sections of the vas deferens at about the middle of its length showed it to be a tube considerably larger in diameter than the largest part of the epididymis. It was lined with columnar epithelium showing, in some sections, two rows of nuclei close together. In some sections cilia could be distinguished, but they were not so easily demonstrated as in the epididymis. There was a subepithelial layer of non-muscular tissue, probably the mucosa, and outside this a thick layer containing smooth muscle fibers. We were unable to distinguish different muscular layers in our sections.

The lumen of the vas deferens and also parts of the epididymis contained masses which stained strongly with eosin. These masses included irregular fragments that took the chromatin stain.

In general, the histological study of the right genital gland led to the same conclusion as did that of the left, namely, that we were dealing with a degenerating structure. As is indicated in the

foregoing description, however, the right gland approached somewhat more nearly to the normal than did the left. Whether this organ was even functional, however, in the sense of containing actively dividing spermatogonial cells, cannot be determined from the evidence afforded by the histology of the gland at death. It may or may not have been. One cannot tell. So then again our results on the question as to whether actual spermatogenesis occurred in this hermaphrodite fowl are negative. All that can be positively affirmed is that at the time when the bird was killed, both sexual glands were in an inactive and degenerating condition.

Discussion of Results.

The case above described presents a number of points of considerable theoretical interest which we may now, with the facts in hand, proceed briefly to discuss.

The first point to which we would direct attention is the peculiar combination or correlation of sexual characteristics (primary and secondary) which existed in this bird. Externally it presented a condition essentially similar to the rarely observed antero-posterior gynandromorphism of insects. Anteriorly the bird was male in its external somatic characters; posteriorly it was female. The truth of this statement may be demonstrated in a striking manner by placing the edge of an opaque card along a line connecting the letters *a* and *b* in Fig. 1 of Plate I. and turning the card about this edge as an axis so as to expose alternately the anterior and posterior parts of the bird. When the card covers the posterior part of the bird what one can see (*i. e.*, the anterior part) is unmistakably and indubitably male. On the contrary, when the anterior part is covered by the card, what of the bird is then to be seen is equally unmistakably female. The "maleness" and "femaleness" of these two portions of the body extend to the most minute details of structure, perhaps not apparent to anyone not perfectly familiar through first-hand practical experience with poultry and particularly Barred Plymouth Rocks. Thus the beak — which is not ordinarily reckoned as a secondary sexual character — in this bird is to the fancier unmistakably that of a male.

It is certainly a remarkable fact that with this perfectly clear

and definite somatic gynandromorphism there is associated an absolutely inactive condition of the primary sexual organs, so far as the functions of spermatogenesis and oögenesis are concerned. The case shows clearly enough that the secondary sexual characters of both sexes *may* exist without the accompaniment of functionating germinal epithelium in the same individual. It does *not* prove that the secondary characters may originally *develop* in the absence of the functioning of the primary glands, because of the uncertainty as to whether either of the glands was ever functional in this specimen.

There has accumulated in recent years a considerable mass of evidence,[1] particularly from medical, surgical and gynecological workers, tending to show that the development of secondary sexual characters is in some way controlled through internal secretions (containing hormones) produced in some part or parts of the primary sexual apparatus. While the general fact of such a relationship is now to be regarded as quite definitely established, the details of the process are as yet by no means worked out. Whether these secretions are elaborated in cells of the germinal epithelium proper, from interstitial or stromal cells, or from the accessory parts of the reproductive apparatus (*e. g.*, epididymis, oviduct, etc.) is, in general, still unknown. It might at first thought be supposed that the present case, inasmuch as the glands are degenerate and non-functional whereas the accessory male and female organs (epididymis, vas and oviduct) are complete and normal, afforded evidence in favor of the view that these latter organs are sources of internal secretions influencing secondary sexual characters. Any presumptive warrant for such an inference, however, is largely if not entirely taken away by evidence of another kind. We have conclusively shown, for example, in unpublished experimental work that complete or partial removal or ligation or section of the oviduct in the domestic fowl, undertaken before or after the oviduct has become functional, is without any effect whatever on the development or

[1] It seems unnecessary to print *in extenso* here the long list of literature which exists on this subject. An introduction to this literature will be found in Morgan's "Experimental Zoölogy," Chapters 28 and 29, and in Bayliss, W. M., and Starling, E. H., "Die chemische Koordination der Funktionen des Körpers," *Ergeb. der Physiol.*, Jahrg. V., pp. 664–697, 1906.

persistence of the female secondary sexual characters. The fact that in man vasectomy (practised, for example, in Indiana for the sterilization of criminals and certain other undesirable citizens) produces no effect whatever on secondary sexual characters or the sexual appetite is again evidence in the same direction.

The present case, of course, affords no direct evidence as to whether a secretion influencing secondary sexual characters may not be produced by the interstitial or stromal cells.

A further point of considerable interest lies in the fact that in this bird we have a fully developed, normal, and so far as can be told, entirely functional oviduct in the absence of a functional ovary. Normally in the hen the oviduct is in an atrophied, non-functional condition at times when laying is not going on, *i. e.*, when the ovary is not functioning. In the young pullet the oviduct stays in an infantile condition until the oöcytes begin to enlarge by the deposition of yolk just before laying begins. As the yolks approach the size at which they are separated from the ovary the albumen-secreting and other glands of the oviduct become enormously enlarged and the whole organ passes into the "laying condition." After laying stops the glands quickly atrophy and the whole organ goes back to the adolescent condition. In other words, there is in the normal bird a close correlation between the functioning of ovary and oviduct. There is, of course, a similar apparent correlation between ovary and uterus in mammals.[1] Now in this hermaphrodite specimen the correlation is apparently upset. We have the oviduct in "laying condition" in a bird in which the ovary is absolutely non-functional so far as ovulation is concerned. The two cases of hermaphroditism in the domestic fowl described by Shattock and Seligmann[2] essentially parallel ours in this regard. In both cases they found a well-developed

[1] Here the brilliant work of Dr. Leo Loeb is establishing, by means of analytical experimentation, the causal factors in the physiology of the uterus. Cf. Loeb, L., "The Production of Deciduomata and the Relation between the Ovaries and the Formation of the Decidua," *Jour. Amer. Med. Assoc.*, Vol. L., pp. 1897–1901. June 6, 1908.

[2] Shattock, S. G., and Seligmann, C. G., "An Example of True Hermaphroditism in the Domestic Fowl, with Remarks on the Phenomenon of Allopteratism," *Trans. Pathol. Soc. London*, Vol. 57, pp. 69–109, Plate I., 1906. "An Example of Incomplete Glandular Hermaphroditism in the Domestic Fowl," *Proc. Roy. Soc. Medicine*, Vol. I., pp. 3–7, 1907.

oviduct, though the ovary was distinctly not in *ovulating* condition.[1] These cases point strongly to the idea that the mutual interrelationship between ovary and oviduct in birds is very far from being of such a simple character as one would be led to infer from observation of normal specimens. Here, as in other instances, teratology may furnish the clue for the elucidation of a normal physiological process.

Summary.

The purpose of this paper is to describe in detail a case of incomplete hermaphroditism in the domestic fowl. It is shown that:

1. In its external somatic characters the specimen was an antero-posterior gynandromorph.

2. Internally the bird possessed on the left side a large, lobulated gland in the position and anatomical relations normal to the ovary. There was also a fully developed, normal oviduct, in functional condition on the left side of the body.

3. On the right side of the body was a small organ in the position and anatomical relations normal to the right testis. Attached to this organ was a normal epididymis and vas deferens leading to the cloaca.

4. Microscopical examination showed that both sex glands were in a condition of extreme degeneration. Neither spermatogenesis or oögenesis could be found in any part of either gland.

5. Certain theoretical aspects of the case are discussed.

[1] These authors did, in these cases, succeed in finding some evidence of actual oögenesis, but not of ovulation, either past or prospective.

Explanation of Plate I.

Showing the hermaphrodite specimen described in the text. A normal male and female of the Barred Plymouth Rock breed are shown for comparison.

Fig. 1. Hermaphrodite specimen. A line connecting the letters *a* and *b* marks the division region between the male and female portions of the gynandromorphic condition. Cf. text, p. 280.

Fig. 2. Normal Barred Plymouth Rock cockerel.

Fig. 3. Normal Barred Plymouth Rock pullet.

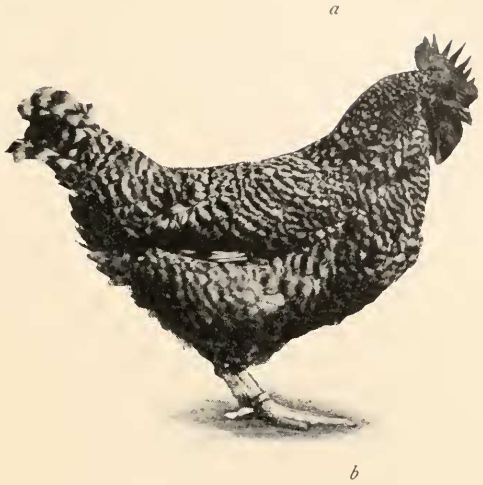

FIG 1

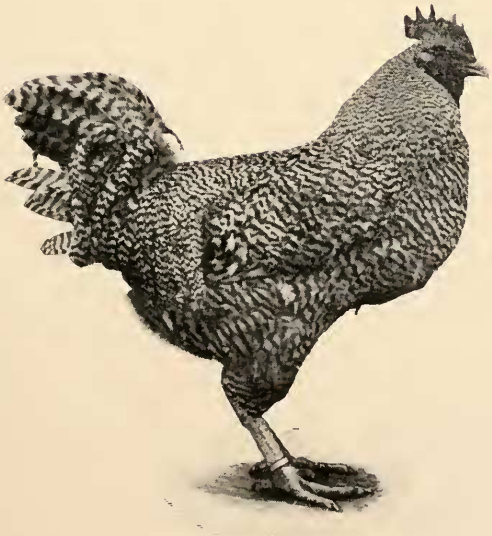

FIG. 2.

FIG. 3.

Explanation of Plate II.

Fig. 1. Photograph showing the gross anatomy of the genital organ of the hermaphrodite specimen. *O*, ovary. *F*, funnel mouth of oviduct (ostium tubæ abdominale). *S*, region of shell gland of oviduct. *T*, testis. *V.D.*, vas deferens. *U*, right ureter. A black card is placed behind the vas deferens and ureter in the lower portion.

Fig. 2. Microphotograph of section through epididymis. Obj.: Spencer 32 mm.; 6 × compensating ocular.

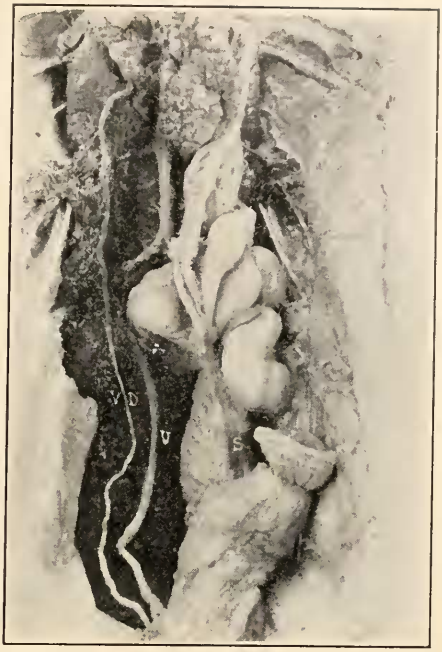

FIG. 1.

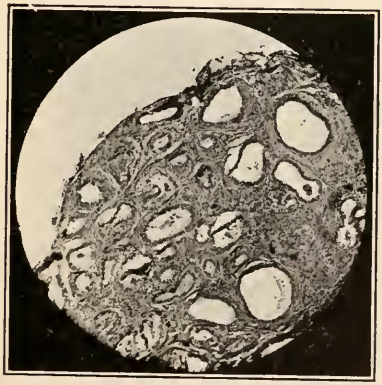

FIG. 2.

FURTHER STUDIES ON THE LIFE CYCLE OF PARAMECIUM.

LORANDE LOSS WOODRUFF.

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I. Introduction.

The life cycle of infusoria has been the subject of numerous investigations since Ehrenberg suggested on *a priori* grounds that the protozoa are so simply organized that they are not subject to natural death, and Dujardin opposed the view and maintained that the life history of infusoria comprises a cyclical change in vitality which terminates in death.

Bütschli ('76), Engelmann ('76), Maupas ('88; '89), Joukowsky ('98), Simpson ('01), Calkins ('02; '04), Woodruff ('05), Popoff ('07) and Gregory ('09) have all advanced evidence tending to show that infusoria when bred under somewhat constant culture conditions pass through a more or less definite physiological cycle. This cycle is characterized by an initial high potential of division which gradually is expended until reproduction finally ceases, and death puts an end to the cycle unless conjugation is permitted or artificial stimuli are employed. Characteristic morphological changes, both cytoplasmic and nuclear, appear in many cases as "senile degeneration" increases.

Enriques ('08) in a recent paper has again opposed the idea of old age and physiological death in protozoa and has contended that the results which support the cyclical character of the infusorian life history have been obtained by faulty culture methods. The conclusion of Enriques is, I believe, somewhat too sweeping, and is based in part on a misunderstanding of the methods by which the most extensive cultures have been con-

ducted. The work on the infusorian life history has clearly shown that many species of infusoria, when bred on a more or less constant culture medium, pass through quite definite cycles. Calkins, Woodruff, and Gregory have shown also that specific changes in the environment at critical times may "rejuvenate" a culture and lengthen its life for long periods. It is demonstrated, I believe, that the life history of the infusorian is cyclical when subjected to a constant environment, and it is also demonstrated that the life history may be lengthened by the timely use of various stimuli.

I have defined a cycle as "a periodic rise and fall in the fission rate, extending over a varying number of rhythms, and ending in the extinction of the race, unless it is 'rejuvenated' by conjugation or *changed environment*."[1] This suggests the idea that it may be possible to eliminate the cyclical character of the division rate by *constantly* subjecting the organisms to a varied environment and the present investigation is devoted to this aspect of the problem. In a former paper[2] I have given an outline of my studies up to May, 1908, on the life history of *Paramecium* when subjected to a varied environment. The present paper presents the data to June 29, 1909.

II. Methods.

A "wild" *Paramecium aurelia* (*caudatum*) was isolated from a laboratory aquarium on May 1, 1907, and placed in about five drops of culture medium on an ordinary glass slide having a central ground concavity. When this organism had divided twice, producing four individuals, each of these were isolated on separate slides to start the four lines, *I–a*, *I–b*, *I–c* and *I–d* which compose this culture (Paramecium I).[3] The culture has been continued by the isolation of an individual from each of these lines almost daily throughout the life of the culture up to the present time (June 29, 1909). A record has been kept of the daily divisions of each line, and the average rate of division of the four lines of the culture and this again averaged for five- ten- and thirty-day

[1] Woodruff ('05).

[2] Woodruff ('08[2]).

[3] For further details in regard to technique see Woodruff ('05).

periods has been plotted (cf. Figs. 1, 2 and 3). Permanent preparations have been preserved at various periods in the life history for the purpose of studying the cytoplasmic and nuclear changes, if present.

The culture was carried on at the Thompson Biological Laboratory of Williams College, Williamstown, Mass., during May and June, 1907; at the Marine Biological Laboratory, Woods Holl, Mass., during July and August, 1907 and 1908; and at the Sheffield Biological Laboratory of Yale University, New Haven, Conn., from September, 1907, to July, 1908, and from September, 1908, to the present time (June, 1909).

During the first nine months of the work the culture medium was made of hay or grass; but, except during certain periods in which the culture was employed as a control for special experiments,[1] the infusion was made with hay from various localities, and different proportions of hay and water were used almost daily. Water from different sources was employed. The temperature of the infusion was always raised to the boiling point. In some cases the infusion was used as soon as it had again attained the room temperature; in others, it was allowed to stand for twenty-four hours before it was employed.

From February, 1908, to the present time, June, 1909, however, a more varied culture medium was employed. *Paramecium* will thrive in nearly any infusion which may be made from materials collected in ponds and swamps, and therefore, in an endeavor to supply as far as possible all the elements which may be encountered in the usual habitat of the organism, water was taken from ponds, laboratory aquaria, etc., together with their animal and plant life. In other words, no definite method was used in selecting the material, but it was simply collected at random from what might be the abode of infusoria, and thoroughly boiled. Probably the only condition present in the life of this culture which could not be encountered by a wild *Paramecium* was that the water had been boiled, but this was essential in the experiments in order to obviate the possibility of the contamination of the culture by an active or encysted wild specimen. Conjugation was impossible in the direct lines of the culture on account of the frequent isolations and change of medium.

[1] Woodruff ('08[1]).

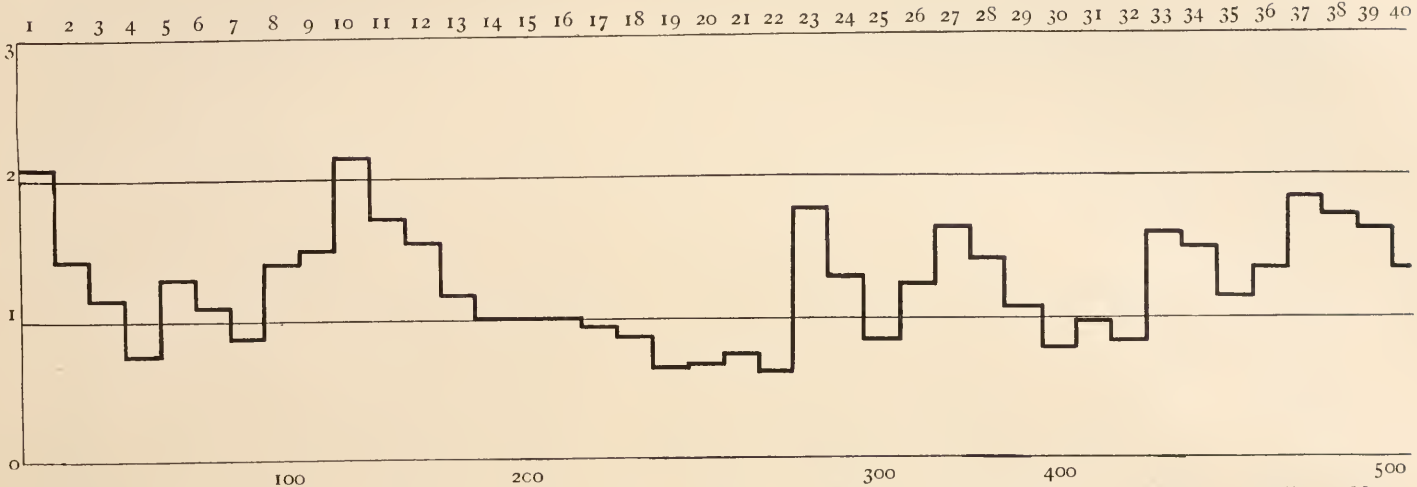


DIAGRAM I. PART I.

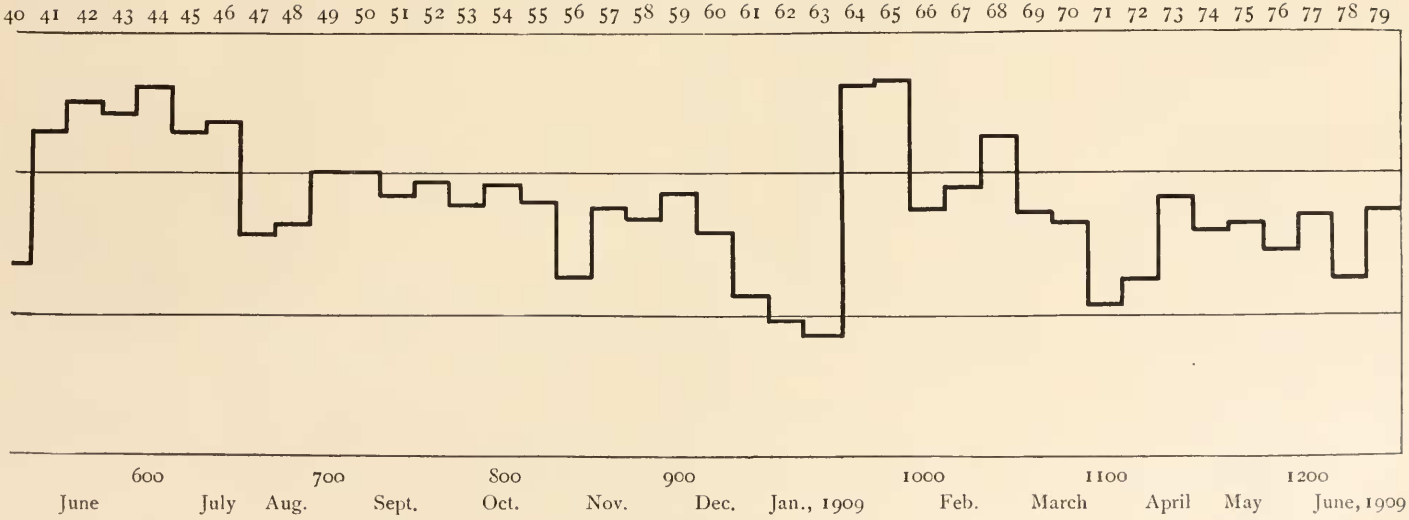


DIAGRAM I. PART 2.

FIG. 1. (Ten-day periods.) Complete history of *Paramecium aurelia* (*caudatum*), Culture I, from start on May 1, 1907, to the present time, June 29, 1909, at the 1,238th generation. The rate of division is averaged for ten-day periods. The ordinates represent the average daily rate of division of the four lines of the culture. Above, the numbers of the ten day periods are indicated. Below, are designated the periods in which the fifteenth day of the respective months fell. The figures, 100, 200, etc., represent generations and are placed in the periods in which they were attained.

III. Description of Cultures.

Culture I has attained, during the first twenty-six months of its life, the 1,238th generation. The average rate of reproduction for the entire period has been over one and a half divisions per day, and during not a single ten-day period has the average rate fallen as low as one division in two days, while during several ten-day periods the rate has averaged over two and a half divisions per day.

Fig. 1 shows, by the familiar block method, the average rate of division of Culture I for ten-day periods. Especial emphasis is put on the character of this curve as the results of Calkins' cultures are plotted for ten-day periods. A study of this diagram shows that the life history falls naturally into two parts. The first extends from period one to period thirty, and the second extends from period thirty to the present time. These two major parts of the curve are coextensive with different methods in the treatment of the culture. The culture medium used during the periods covered by part one was very much more uniform than that used during part two; the decidedly varied environment not being employed until February, 1908. The effect of this treatment is shown in the decided change in the character of the curve of the division rate from that period to the present. The general vitality of the protoplasm is considerably higher as is shown by the fact that only once since that time has the curve for a ten-day period fallen below one division per day, and that period represents the culmination of the more or less severe treatment which the culture received when it was carried to Baltimore during convocation week. However, to the change of water and other conditions incident to the journey is apparently to be attributed the stimulus which enabled the culture to attain a short time after an average rate of nearly two and three quarters divisions per day (see Fig. 1, periods 64 and 65), the highest reproductive power shown in the life history for any ten-day period.

A study of Fig. 2, which is plotted by the same method as Fig. 1 except that the periods are of thirty-day instead of ten-day duration, also shows clearly the effect of the varied culture medium which has been in use from February, 1908. The rate

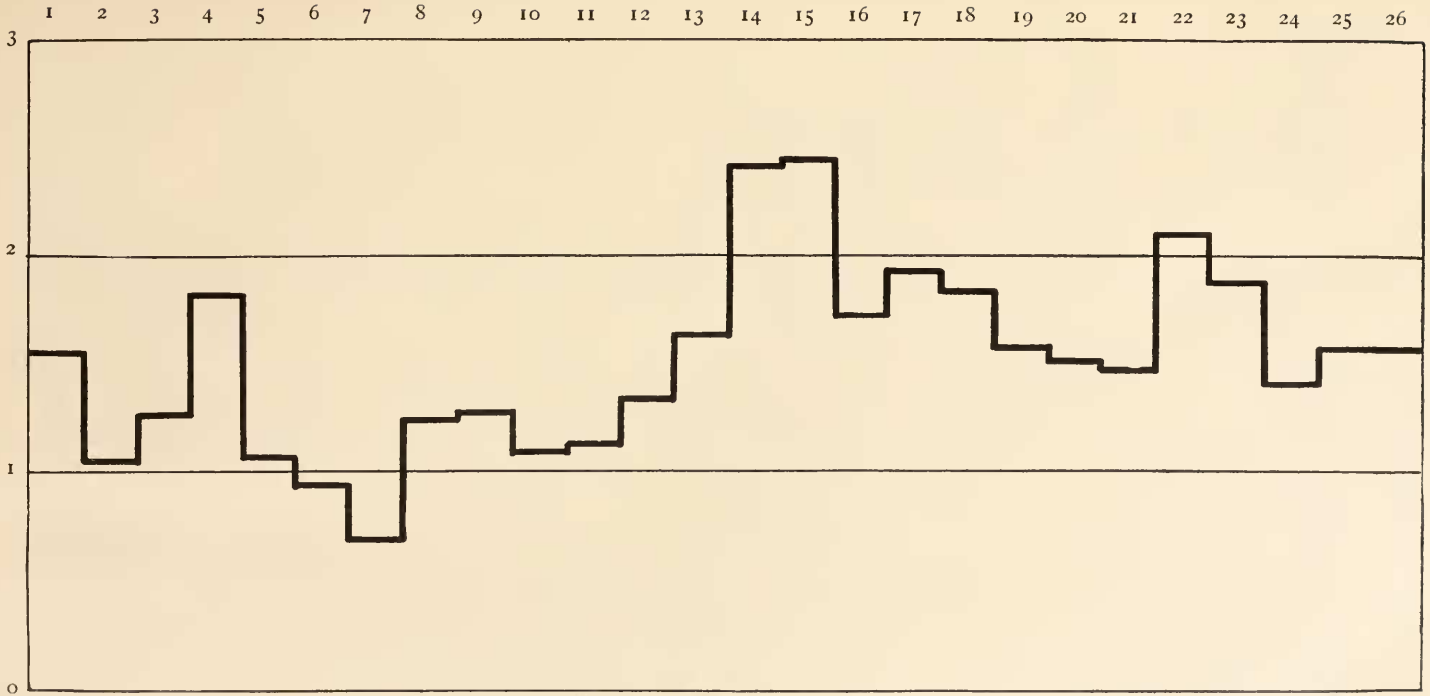


DIAGRAM II.

FIG. 2. (Thirty-day periods.) Complete history of *Paramecium aurelia* (*caudatum*), Culture I, from start on May 1, 1907, to the present time, June 29, 1909, at the 1,238th generation. The rate of division is averaged for each month of the life of the culture. The ordinates represent the average daily rate of division of the four lines of the culture.

of reproduction has never again fallen as low as it was when this treatment was begun. When averaged for thirty-day periods the highest rate of division appears in June and July, 1908; the great rise in vitality which occurred in February, 1909, and is shown in periods 64 and 65 of Fig. 1 (ten-day periods) is not so conspicuous, as the average is reduced by the low rate of fission during the two periods preceding.

A similar examination of Fig. 3, in which the rate of division is averaged for five-day periods, is not so instructive because the influence of the rhythms is more clearly brought out during short periods, so that the general trend of the curve of the life history is somewhat obscured. However, when the curve is surveyed in its entirety it illustrates the fact that the vitality of the organisms, as indicated by the fission rate, has maintained a higher average since the use of a promiscuous culture medium was instituted.

In order to determine more fully the effect of a very constant environment on this same race of *Paramecium* which was being maintained on a varied environment, there was isolated from each of the four lines, on February 19, 1909, at the 1,121st generation, a second culture, designated *Paramecium* I^s. This culture was submitted to as constant an environment as was practicable, according to the general method of Calkins. There were, then, two cultures of the same protoplasm running simultaneously, one being subjected to a varied or promiscuous culture medium, and the other to a comparatively constant culture medium. As a matter of precaution, and to show if there was anything intrinsically deleterious in the medium provided for Culture I^s, its constant medium was employed at various times as a temporary medium for Culture I. This, of course, simply increased the variability of the medium of Culture I. Also, near the end of the I^s series, its medium was employed not only for Culture I, but also for two cultures of ex-conjugant paramecia (*Paramecium* II^y and *Paramecium* II^z) from an entirely different source from that of the *Paramecium* of Culture I.

The results of these experiments with a constant and varied environment on the same protozoan protoplasm are shown graphically in Fig. 4. A glance at this curve shows that the vitality of the protoplasm of Culture I^s (constant environment), as meas-

ured by the fission rate, immediately fell below that of Culture I (varied environment), and that a consistent decrease in division rate was maintained until Culture I^s died out on June 6, 1909, at the 1,159th generation, after having been one hundred and seven days, or a little more than three months, on the constant medium; whereas the protoplasm of Culture I maintained about the same general average vitality throughout the period and had attained the 1,200th generation, a gain of forty-one generations in 107 days over the I^s culture. That the death of the I^s culture was not due to some sudden and accidental inimical change in the medium is proved by the fact that the same culture medium when used temporarily for the other cultures produced no deleterious effect, and also by the character of the curve of the fission rate of the I^s culture which has a consistent general downward trend except as it is affected by the rhythms. A comparison of the I^s culture curve and the curves of Calkins' *Paramecium* cultures shows a striking similarity in character. The cycles in Calkins' A culture were of six months duration and varied between 126 and 200 generations in length. My I^s culture passed through only 138 generations, but as it actually represents only the downward slope, or second half, of a cycle of Calkins' culture, my I^s cycle is really somewhat longer than those of Calkins. This point is only of interest in that it indicates in a general way the comparative similarity of the reactions of the protoplasm of paramecia from widely different sources to the same general conditions; and because it removes the possible objection that the I^s culture died out because it had been acclimated to the varied environment, and consequently it could not withstand the change to a constant medium. Of course, this is only a formal objection at best as there is every reason for supposing that the wild paramecia with which all cultures are started have been subjected for countless generations to considerably greater variations in their environment than it is possible to supply artifically.

IV. Discussion.

Up to the present time Culture I has not completed a "cycle" and all the fluctuations in vitality, as indicated by the division rate, fall under the head of "rhythms," as previously defined by me,[1]

[1] Woodruff ('05).

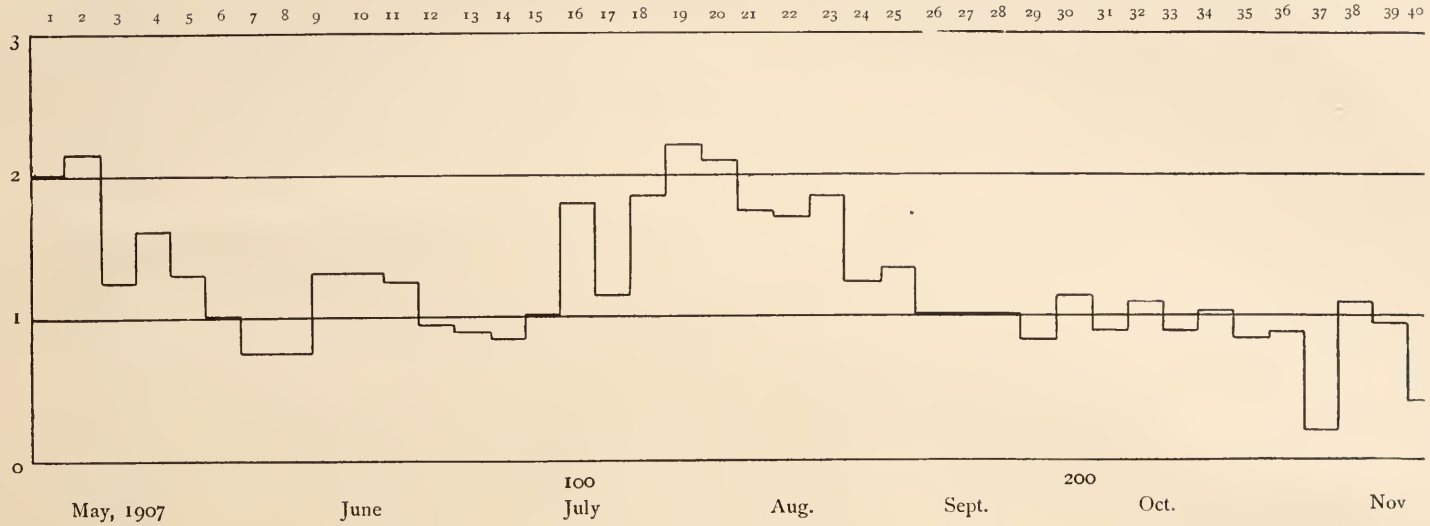


DIAGRAM III. PART 1.

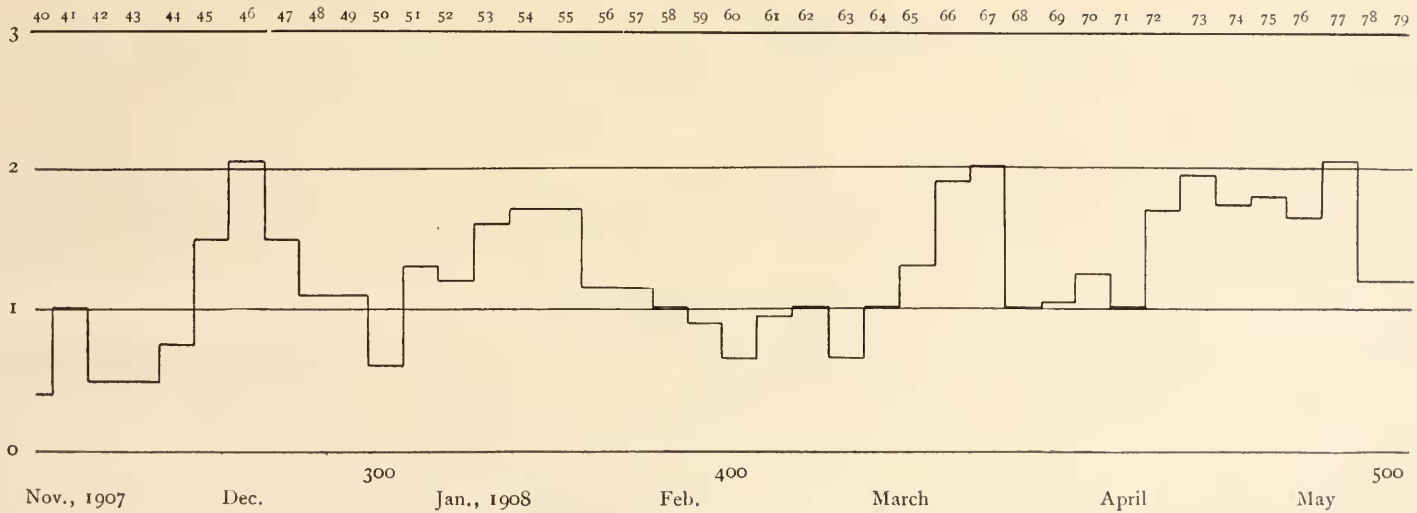
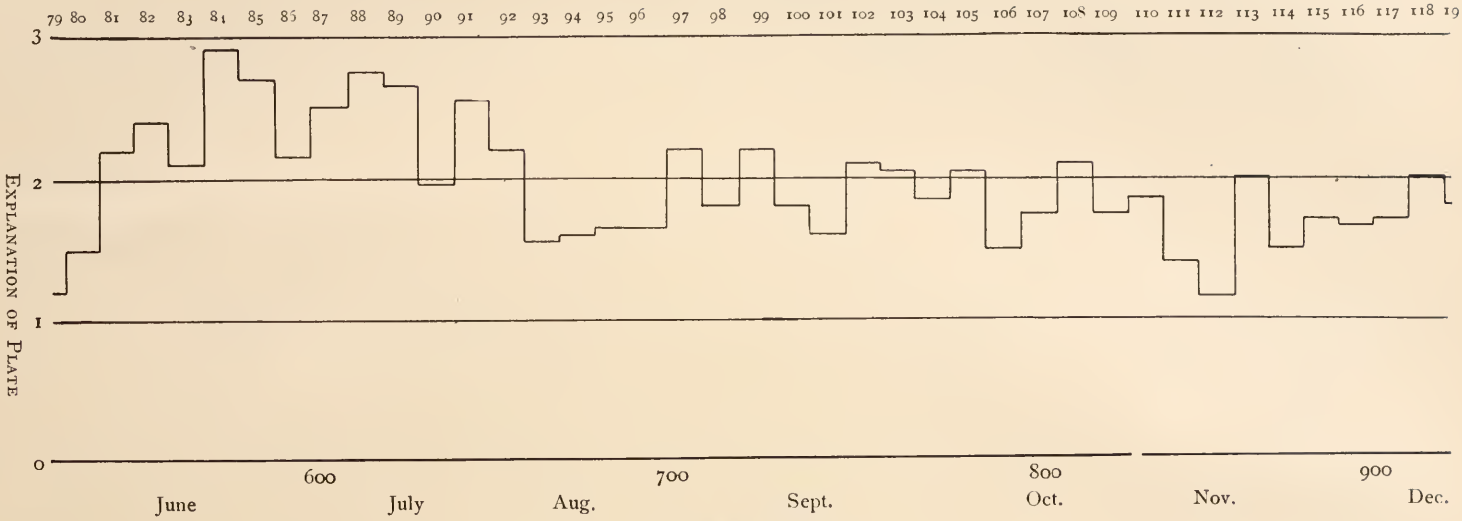


DIAGRAM III. PART 2.



EXPLANATION OF PLATE

DIAGRAM III. PART 3.

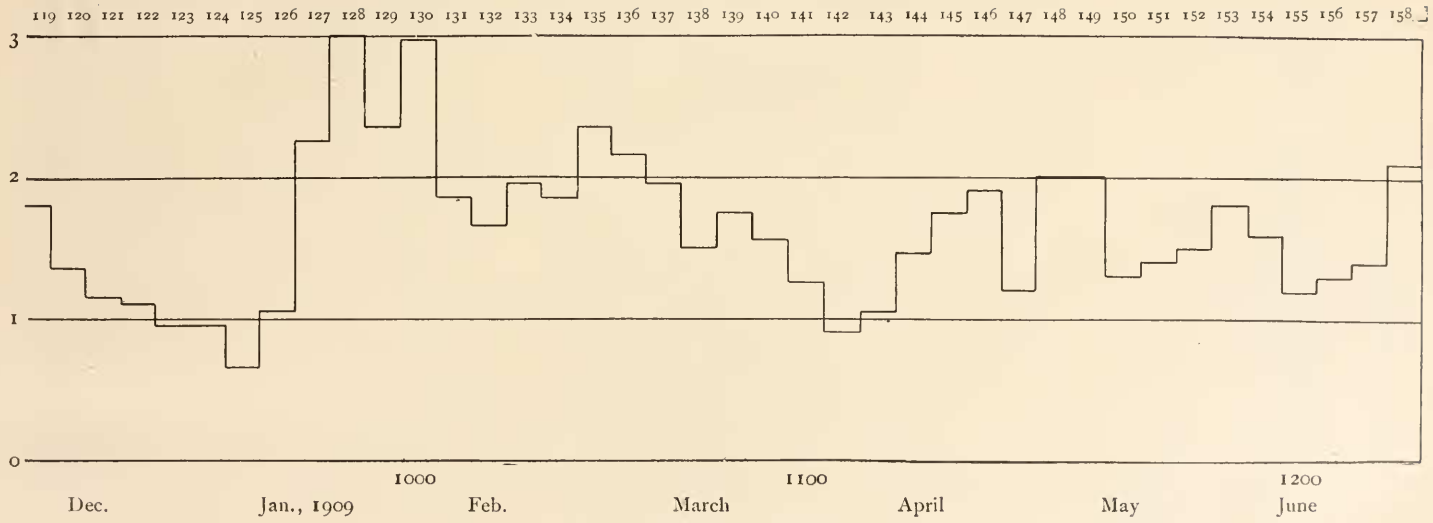


DIAGRAM III. PART 4.

FIG. 3. (Five-day periods.) Complete history of *Paramecium aurelia* (*caudatum*), Culture I, from start on May 1, 1907, to the present time, June 29, 1909, at the 1,238th generation. The rate of division is averaged for five-day periods. The ordinates represent the average daily rate of division of the four lines of the culture. Above, the numbers of the five-day periods are indicated. Below, are designated the periods in which the fifteenth day of the respective months fell. The figures, 100, 200, etc., represent generations, and are placed in the periods in which they were attained.

viz., "A rhythm is a minor periodic rise and fall of the fission rate, due to some unknown factor in cell metabolism, from which recovery is autonomous." The rhythms are more evident when a more constant environment is maintained, as I have shown in a study of the effect of a particularly stable environment on *Gastrostyla steinii*, during the months of July, August and September (cf. Fig. 5).

Gregory ('09) has plotted the curve of a *Stylonychia* culture for five-day periods from the data of Popoff ('07), which shows that the first four of the so-called "deep depression" periods emphasized by Popoff resolve themselves into "normal rhythms from which recovery is autonomous." Gregory also points out in her own 548 generation culture of *Tillina magna* that "the curve which represents the general vitality of the protoplasm shows the normal rhythmic fluctuations observed by Woodruff."

I have previously interpreted as rhythms the tri-monthly depressions in vitality, which Calkins and the earlier workers on *Paramecium* have noted, and the results obtained from my culture of *Paramecium* seem to indicate that the semi-annual cycles of Calkins are also actually rhythms, recovery from which was not autonomous under the conditions of a constant environment. The general occurrence of rhythms in the life history of infusoria is established, I believe, but to what they are due is still awaiting discovery.

Gregory has emphasized the point that "Enough consideration has not been taken of the fact that not only does each individual vary in its degree of sensitiveness at different periods in the life history, as suggested by Towle and shown by the rhythms of Woodruff, but each individual of the same species as well as of different species has its own peculiar protoplasmic reactions. Woodruff himself has failed to consider this fact in his last paper on the effects of a varied environment on *Paramecium*. . . . He cannot logically compare his results with those of Calkins for he is not dealing with the same protoplasm. . . ." In 1905 I wrote: "My cultures lead me to believe, with Simpson, that the personal equation, if I may use that term, of the individual selected to start a culture has the most influence in determining the number of generations attained. . . . Calkins' discovery of

what he calls 'incipient fertilization' . . . would seem to bear out this point, and to show that the number of generations, or the period, over which a cycle extends, is not a point of great moment."

I have since found no reason to alter this opinion. But there must be *limits* beyond which the "peculiar protoplasmic reactions" of any individual do not extend, otherwise each would be a law unto itself and there would be as many laws as individuals. Certainly we may reasonably assume that there are limits of time, and generations, which a "cycle" (if it exists) of any particular species will not exceed. The earlier investigations apparently indicated that about three months or about 100 generations was the limit of the cycle of *Paramecium*. Calkins in his last paper extended the cycle to about six months, or about 200 generations. The present culture extends the "cycle" to more than twenty-six months, and more than one thousand two hundred generations. The longest culture carried by Calkins (Culture A) lived for twenty-three months, and attained 742 generations — but this comprised four complete cycles, the last one terminating fatally. It is necessary to contrast the cycle of Calkins' culture of about six months duration, and two hundred generations, with the life (cycle) of this culture, which is of twenty-six months duration at present, and 1,238 generations. That is, this culture shows a "cycle" twenty months longer in time, and, so far, of over one thousand more generations.

The *character* of the life history must also be taken into account. There is a marked difference in the character of the *Paramecium* curve after February, 1908, when the decidedly varied environment was begun (cf. Figs. 1 and 2). A similar difference in character is evident in the *Gastrostyla* culture when the more constant medium was being maintained (cf. Fig. 5, July, August and September), and the same is again strikingly shown in the present culture of *Paramecium* in the experiments which subjected the "same protoplasm" to a constant and a varied environment simultaneously (cf. Fig. 4).

The term cycle, as has been pointed out, is a relative one, but I think it is necessary to extend the conception of the cycle (as worked out on infusoria on constant media) to an unwarranted

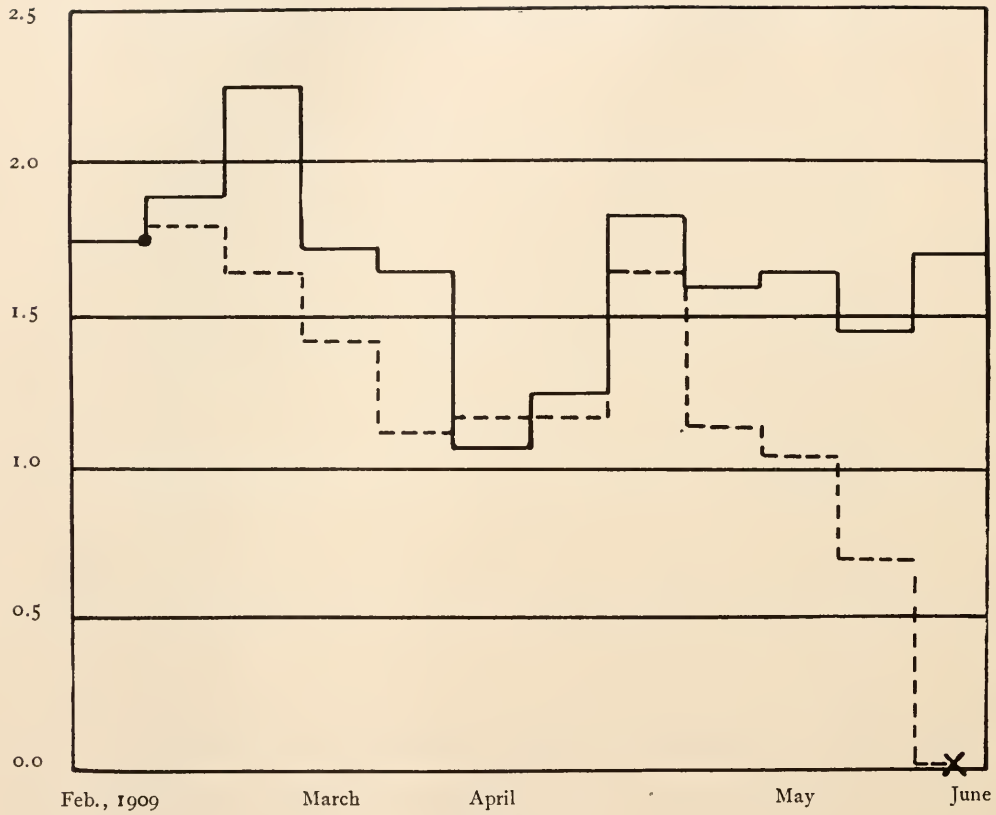


DIAGRAM IV.

FIG. 4. *Paramecium*, Culture I^{s} (constant environment) = broken line; Culture I (varied environment) — continuous line. ● = point of isolation of Culture I^{s} from Culture I. *X* = point at which Culture I^{s} died out. Other details as in Fig. 1.

extent if it is to be made to include the life history of the present culture. I would not suggest that the protoplasm of every wild *Paramecium* has the potential to attain over twelve hundred generations or more — undoubtedly there are strong and weak strains among infusoria as among other classes of animals. Again, it is possible that the different races of *Paramecium* which Jennings ('08) has been able to isolate may have a physiological as well as a morphological basis of distinction. It may be also that I have been particularly fortunate in my haphazard selection of culture material, so that the proper variations have been available when necessary. It is true that any particular ingredient of the infusion which might be needed would not long be available for the organisms on account of the frequent change of the character of the infusion, and Gregory has recently shown in the case of *Tillina*, and I have shown in the case of *Gastrostyla*, that daily stimulation with salts is often more efficacious than an initial stimulation. But Calkins says in regard to the second cycle of his A culture ". . . in December it was necessary to keep them on the stimulant only a day or two to get the desired result. The short treatment at this period sufficed, because they were not allowed to become weakened to the same extent as in the preceding period of depression." It is this factor which has been taken into account in this study, and it is probable that it has contributed largely to the vitality of the culture.

It must also be kept in mind that a certain amount of judgment is exercised in selecting a representative specimen for isolation. By experience one becomes quite familiar with the normal movements, shape, and general appearance of the organisms, so that it is possible to select a favorable specimen daily for the continuance of each of the lines. The precaution is nearly always taken to examine the culture again a few hours after the isolations to see how the organisms behave in the fresh culture liquid. If everything does not appear normal, a new set of individuals is isolated from the "stock" (*i. e.*, from the one, three or seven individuals left after isolation, the number depending on the rate of division during the previous twenty-four hours). Undoubtedly the process practically results in the artificial selection of the organisms which have the highest poten-

tial of division and those which are most readily acclimated to changes in their medium. Each and all of these factors may contribute to the length of the life of the culture — but after all is done the "chances" are largely against the prolonged life of the culture.

This culture suggests, then, the time-honored question whether the protoplasm of infusoria has the potential of unlimited life and reproduction, and the fundamental question as to the rôle of conjugation in the life history of these organisms. Up to the present time there has been no tendency to conjugate among the individuals of this culture, although in the "stock" cultures, consisting of the individuals remaining over after the daily isolations, there has been ample opportunity for it to take place. The daily isolations, of course, have precluded its occurrence in the four direct lines of the culture. This result agrees with those of Joukowsky on a 460 generation culture of *Pleurotricha lanceolata*, Gregory on a 548 generation culture of *Tillina magna*, and Woodruff on an 860 generation culture of *Oxytricha fallax*, on a 448 generation culture of *Pleurotricha lanceolata* and on a 288 generation culture of *Gastrostyla steinii*. Maupas secured no conjugations in his cultures of *Stylonychia mytilus* and *Oxytricha* sp., though his other series yielded plenty of syzygies. That the infusoria do conjugate is, of course, a matter of common observation; but I believe these results indicate that the phenomenon is not so frequent in the life history as is generally believed. A daily examination of twenty hay infusions, made up by several different methods, has not shown a single case of conjugation among the hypotrichous forms present either at the top or bottom of the jars. In fact, not a single syzygy has been observed in any species except *Paramecium*, and in this form conjugation has been very rare. However, a sudden transference of the paramecia from the comparatively constant culture medium of a hay infusion to a different medium has produced marked epidemics of conjugation. It is just possible that a constant medium is necessary for the so-called miscible state (Calkins) to develop, and that this becomes functional on transference to a decidedly different medium. If this is so, it may account for the absence of conjugation in my paramecia series on a varied medium, and

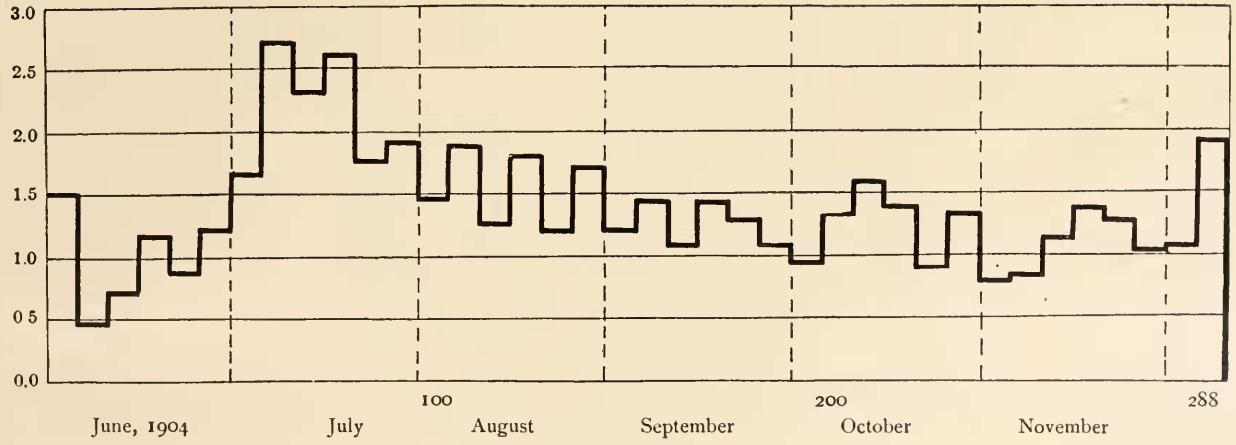


DIAGRAM V.

FIG. 5. Complete history of *Gastrostyla steinii*, Culture A, averaged for five-day periods. Method of plotting is the same as in previous diagrams.

its prevalence in Calkins' cultures on a constant medium. This idea is not supported by my hypotrichous cultures which were carried on a constant medium and still did not develop a tendency to conjugate even when the culture medium was varied in some special experiments. It is highly probable, however, that the superficial conditions which induce conjugation may vary in different species.

Periods of marked physiological depression have not appeared during the first twenty-six months of the life of the *Paramecium* I culture, but well-defined morphological changes have taken place. I shall not discuss these cytological changes at present, as I believe it is advisable to wait until the culture is terminated naturally, or by accident, so that all the data from the complete series may be discussed in its entirety. It is clear, however, that the relation of the rate of division to the so-called "normal" condition of the nuclei of *Paramecium* is not supported by this culture, as decided nuclear changes apparently do not affect the general vitality of the organisms. It may be noted further, that not a single monster due to incomplete or otherwise abnormal division has occurred in the entire 1,238 generations.

V. Conclusions.

The experimental study of the life history of infusoria has so far clearly shown that:

The protoplasm of these organisms, when subjected to a comparatively constant culture medium, passes through long cyclical changes in vitality which finally result in the death of the organism.

The protoplasm may be "rejuvenated" by suitable changes in the culture medium (stimuli) at critical points in the cycle, and thus be enabled to resume active reproduction for a longer period.

The essential fact brought out by this study is that:

The protoplasm of the individual *Paramecium* isolated over two years ago to start the culture has had the potential to divide (so far) over one thousand two hundred and thirty times at an average rate of more than three divisions every two days, and the representatives of the untold millions of its progeny which are

still in captivity give every indication of being in as normal physiological and morphological condition as their ancestor. This suggests that when the protoplasm is constantly subjected to a suitable varied environment the cycle may be greatly prolonged and probably entirely eliminated—the fluctuations in vitality not transcending the rhythm.

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ON THE METHOD OF CELL DIVISION IN TÆNIA.[1]

A. RICHARDS.

Within the last few years the question of the significance of amitosis has pushed its way forward with renewed activity. From the classic views of Ziegler and vom Rath, Flemming and others that amitosis may be expected in unicellular organisms, in degenerating and senescent cells, and in highly specialized and pathological tissues opinion in some quarters has departed widely.

On the one hand the tendency has been to narrow this view. In many of the Protozoa mitosis has been found quite general, at least in some stage of the life cycle, while in certain Rhizopods, as *Arcella* and *Euglypha*, where direct division was formerly thought to be the means of reproduction, it is now known that mitosis is the common method. In the case of highly specialized cells numerous examples are reported in which careful study has shown mitosis as the chief means of division. An example of this tendency is seen in Strasburger's work on the tapetum cells. He found that while the period of mitotic division was very short it was sufficient to account for all the observations that had hitherto been explained on the basis of amitosis. Again the application of improved cytological methods of fixing and staining have thrown into disrepute, to a large extent, the old view of the occurrence of direct division in pathological tissues; indeed the phenomenon of reduction has been described in cancer cells. On these lines of research, then, the tendency has been to limit our notions of the rôle of amitosis in nuclear and cell division.

On the other hand more recently a new line of reasoning has been developed, perhaps more rapidly than the facts warrant. This new line, of which Child is the chief, although not the first exponent, is to the effect that direct nuclear division occurs in rapidly dividing cells and in cases in which an orthodromic or acyclic process is involved. In this connection the statements of

[1] Laboratory of Zoölogy, University of Wisconsin, June 4, 1909.

several writers that mitosis may follow amitosis are to be noted in which they cite cases of direct division in the maturation and pre-maturation stages of various forms. To meet these claims we shall doubtless have to revise somewhat our ideas of the meaning of amitosis, but at present the progress toward such a revision seems to have overstepped the bounds of conservatism.

Among the older workers on this line are Meves, Preusse, and Pfeffer. Meves found amitosis to occur in the early stages of spermatozoön formation in *Salamandra* in the autumn followed by mitosis in the spring, but some of these cells have since been shown to take no part in the formation of spermatozoa. Preusse's work has been much quoted in this connection. He found amitosis in the ovaries of Hemiptera. However, a reinvestigation of this case by Gross in 1901 served to bring it under the theory of Ziegler and vom Rath. Gross showed that this method of cell division did occur but much less widely than described by Preusse. Its occurrence is restricted to two kinds of cells, degenerating and secretory; this, of course, proved that he was dealing with a special case under the old theory. Pfeffer's work on *Spirogyra* has been discredited by Nathansohn; in fact, opinion among botanists is decidedly adverse to the view that amitosis may be followed by mitosis in a single nucleus. This opinion is expressed by Strasburger in his recent summary of the individuality question.

Working on the spermatogenesis of the sparrow in 1900, Loisel saw nuclei which began division by amitosis and later continued by indirect division. He says that the amitosis was not a sign of degeneration; but again, he shows that the greater part of certain spermatocytes and spermatids degenerates. To reach a safe conclusion in this case one must needs know the relation between amitosis in the sex cells and degeneration in the sex products. Degeneration on the part of spermatozoa in the Hemiptera has been traced by Morgan and by Miss Stevens to the absence of a single chromosome. If cells lacking a single chromosome degenerate, certainly one would expect degeneration in cases where part of the sex cells had previously divided by as indifferent a method as amitosis seems to be.

Especial importance has been attached to cases of amitosis in

regulatory growth. G. T. Hargitt was the first to suggest this for hydroids. Child's work on *Tubularia* and *Corymorpha* supports this suggestion. In the growth and regulation of *Planaria*, Bardeen and Child have reported amitosis. However, the figures of Bardeen are far from conclusive, and it is very questionable whether they justify the opinion that amitosis is the method of division here. Child has also worked on various other forms among both vertebrates and invertebrates. In several of them his evidence is lacking in some respects. Reference to his work on the cestodes will be made later.

A few workers have described amitosis in the cleavage of the egg and in the early embryonic development of several forms. Hargitt failed to find mitosis of the egg up to the sixteen-cell stage, working on *Clava leptostyla*. Similar results obtain for *Eudendrium* and *Pennaria*. Beckwith, however, has recently shown that his results were due "simply to the fact that the eggs were not obtained at the right time of day. In eggs collected at the proper time (4 to 6 A. M.) there is no difficulty in proving the typical stages of maturation and fertilization." "Maturation and the early cleavages take place by mitosis and not by amitosis." Hickson and Hill have also studied cœlenterate eggs. Hill in his account of *Alcyonium* oögenesis shows that no polar bodies are extruded, no chromosomes are present, the female pronucleus divides irregularly by amitosis and then disappears, and that probably the first cleavage nucleus is formed from the male pronucleus. The evidence is not complete and the case should certainly be reinvestigated. H. L. Osborne described cases of amitosis in the food-ova of *Fasciolaria*. His results have been corrected and enlarged upon by Glaser. The work of Glaser seems to deserve the most careful consideration in regard to this problem; its bearing on the investigation herewith undertaken is only general, however. Further work on embryos has been done by Child, previously mentioned, and by Patterson on the pigeon's egg. The observations of the latter, while much more extensive than those of Child on the chick embryo, are in agreement with them. Stoeckel thought binucleate ova in man are the result of amitotic divisions. Fick's opinion on the subject of amitosis as expressed in his survey of chromosome hypotheses is based

upon the work of Child and Hill and upon his own *a priori* conclusions. He offers nothing new on the problem.

Direct division has been described in other cases of theoretical importance but those mentioned above are perhaps the most significant.

Methods.

My investigation on the problem of amitosis was suggested by Dr. S. J. Holmes, to whom I owe much for direction during the progress of the work. I have received numerous suggestions from various other workers in the University of Wisconsin, all of which are gratefully acknowledged. My thanks are also due Drs. Grove and Meek, of the Pharmacology and Physiology Departments, for their assistance in collecting material.

Specimens of tape-worms were secured chiefly from dogs. A considerable number of cats was examined, but only one furnished material. The specimens were nearly all fixed in Flemming's fluid, which proved quite satisfactory. Those taken from the cat were fixed in Zenker's fluid to be used with Mallory's connective-tissue stain.

A variety of staining methods was used. Flemming's tricolor stain did not give sufficient sharpness of detail to be of much value. Iron hæmatoxylin is in general satisfactory, but it is to be noted that the nuclei do not differentiate as readily as in many other tissues. The fact that they do not decolorize readily and often do not show their contents clearly must be borne in mind when considering the significance of indentations of the nuclear membrane. Delafield's hæmatoxylin decolorized in acid alcohol gave excellent results. My greatest success, however, in staining this material has been by the use of Kernschwarz with Lichtgrün as a counterstain. Lichtgrün is by far the best stain for cytoplasmic structures that I have tried, its only drawback being the ease with which it fades out.

Two genera and three species of tape-worm have been used: *Tænia marginata* (Batsch), *Tænia serrata* (Goeze), and *Dypilidium caninum* (Leuckart). The last of these is the most favorable for cytological work, but I have only a few specimens of that genus. Even here the nuclei are quite small and not entirely satisfactory owing to technical difficulties. I must protest against

the balancing of results obtained from such unfavorable material as that which the cestodes offer against such favorable objects for cytological study as, for example, the Orthoptera. In the cestodes which I have studied, the cells, except the oöcytes, are much smaller than the insect cells, do not stain as readily, and are often obscured by great masses of intercellular material.

Aim.

My aim in this investigation was to obtain definite evidence as to the occurrence of amitosis in cestode tissues. Observations were begun with the hope of bringing into line, in a small measure at least, the account of cell division in this group with the results generally obtained by workers on other forms. Lack of time has prevented the investigation of many of the secondary questions that have arisen. Thus no attempt has been made to give details of chromosome behavior or structure, and the observations have been limited to the method of cell division in the process of oögenesis and in the growth of somatic cells. The discussion, however, includes occasional reference to related questions.

Observations.

Oögenesis. — The female sex cells in the cestodes in question are by far the largest cells in the body. They are in general round with a relatively large nucleus. The cytoplasm is fibrorecticular and to a certain extent granular. Occasionally large dark granules appear, as in Figs. 15, 16 and 17; their nature has not been definitely made out, but they may be yolk nuclei or, perhaps, nothing more than aggregations of smaller cytoplasmic granules. Frequently they serve to obscure the process of mitosis.

No cell organs are located in the cytoplasm of the resting mother cells; but, at some time during the development of the ovarian egg, a mass, probably of yolk, appears there. I have not observed any regularity in the formation of this mass, for some of the early oögonia have it, while it is not present in some oöcytes. The masses, of course, vary in size. Those which are newly formed have a close resemblance with certain stains to a "nebenkern," and, in fact, have been so called. They

were first described by Sommer in 1874, and named by him "Nebendotter." This expression, which has the claim of priority, seems unobjectionable except on the score of bringing a foreign word into English; no suitable translation has been suggested, however. On the other hand, the body is not a true "nebenkern," and to call it by that term is a misuse of the word.

Fig. 1 is an early oögonium from *T. serrata*. Here the "Nebendotter" appears as an egg shaped body of even consistency stained darker than the nucleus although lighter than the

1

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FIG. 1. Oögonium, stained with iron hæmatoxylin, showing "Nebendotter," and nucleus with chromatin reticulum.

FIG. 2. Oögonium showing same structures as Fig. 1, but from a much later generation.

surrounding cytoplasm. Its reactions to various stains deserve metion. With iron hæmatoxylin it stains readily, appearing as a dark homogeneous mass even after a great deal of extraction of the stain. The nucleus and cytoplasm may be entirely decolorized and the "Nebendotter" still show as a dark body, a fact which led to confusion during the early part of my study, as the nucleus was overlooked and the "nebendotter" taken for it. The true state of affairs was not revealed until I had used another method of staining when the appearances which with iron hæmatoxylin had misled me were explained and the structure of the cells became clear. The new reagents were Kernschwarz counterstained with Litchtgrün. Kernschwarz is a weak stain affecting only the nucleus. In my preparations I have seen no trace of it in the cytoplasm or in the "Nebendotter." Lichtgrün stains both nuclear plasm and cytoplasmic structures. The result, then, with this method of staining is as follows: chromatin and nuclear

reticulum, blackish; nuclear plasm, very light green; general cytoplasm, dark green, fibro-reticulated; "Nebendotter," homogenous "cheesy" green. This last appearance is very difficult to describe but is recognized very easily.

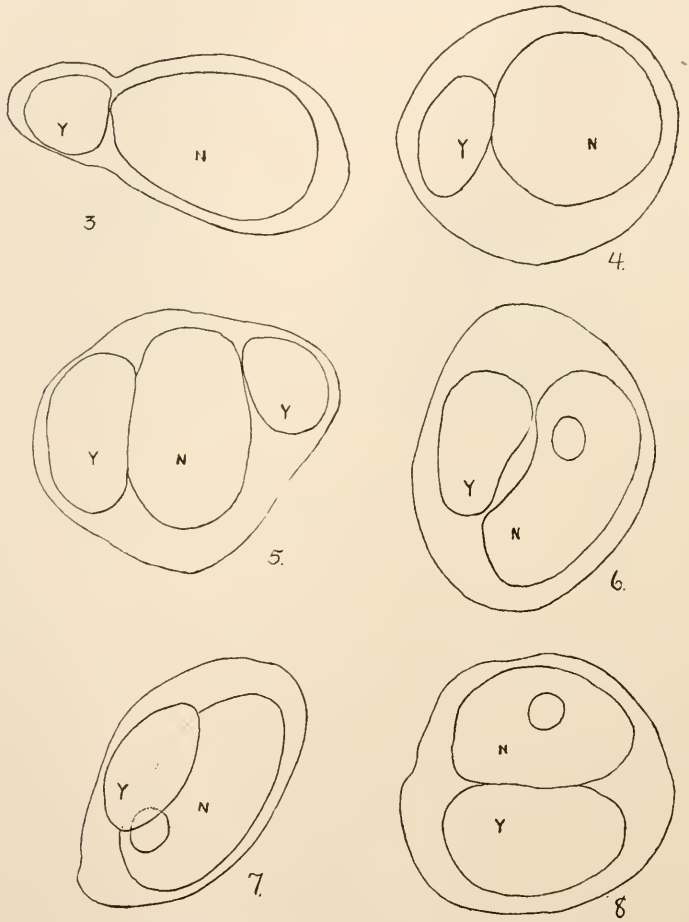
The "Nebendotter" as such has not been described by Child. One is compelled to suspect that the same appearances which confused me may have misled him. Occasionally a constriction is seen in a "Nebendotter" or there may be two or more distinct yolk masses in a cell. More often, especially in the case of hæmatoxylin slides not well decolorized, the "Nebendotter" does not look unlike a dividing or divided half of the nucleus. Child figures cases in which one half of the nucleus stains darker than the other half. Is this darker half perhaps a "Nebendotter"?

To illustrate the above facts a series of outline drawings is given. They were made with the aid of a Zeiss No. 5 ocular and a Leitz one twelfth objective (oil immersion). In each case *n* represents the nucleus and *y* the "Nebendotter." In studying these figures one can easily see how refractive properties may have obscured the boundaries between the various parts. All figures in this series are of resting cells such as are shown in Figs. 1 and 2. Figs. 3 and 4 resemble cases of unequal constriction of the nucleus, Fig. 8, of equal constriction, and Fig. 5 a nucleus divided into three parts by amitosis. Fig. 6 suggests that division began at the center and progressed outward. Compare these figures with those from Child's paper on oögenesis (6): Fig. 1 with his Fig. 29; Fig. 2 with his Fig. 9, *b*; Fig. 3 with his Fig. 8, *b*; Fig. 6 with his Fig. 11, *A*, *a*; Fig. 7 with his Fig. 13, *A*; and Fig. 8 with his Fig. 10, *A*. The similarity is very suggestive.

Resting nuclei are seen in Figs. 1, 2, and 9. Fig. 9 is lacking in a "Nebendotter." The nuclear plasm in the resting condition seems homogeneous throughout and takes a very light stain. The nuclear membrane is a very delicate structure, showing as a thin line in some cases, while in others its location is marked only by the inner edge of the cytoplasmic reticulum. A nucleolus is usually present in the early stages of cell formation; it takes a very light stain in some cases resembling the "Nebendotter." I have never seen a divided or dividing nucleolus.

With regard to their chromatin content the nuclei of *Tænia*

differ from those of *Monieza*. Child states that "the only deeply staining portions of the nucleus up to this time (end of oögonial division period) have been the nucleolus and frequently a few other granules." In another place he says that the nuclei do



FIGS. 3–8. Outline drawings from oögonia showing various relations assumed by the nebendotter and nucleus. *N*, nucleus; *Y*, "Nebendotter."

not contain any definite reticulum. That his statements do not hold for *T. serrata* may be seen from my Figs. 1, 2 and 9. The chromatin content is small in amount, but it can be seen in definite masses which are scattered over the periphery of the nucleus and which are connected by definite strands of linin. The reticu-

lar character of the chromatin and linin is clearly shown with Kernschwarz and Lichtgrün. This description answers not only for late oögonia but also for the early ones and for the oöcytes. Fig. 9 is an early oögonium.

Fig. 10 illustrates the early condition of the spirem stage. Oögonia in this stage and slightly later are very numerous in certain lots of material. In other lots I find many resting stages. Anaphases and telophases, too, are not difficult to find but metaphases are conspicuously absent. This probably means that the metaphases are of short duration. It also indicates a fact which I believe to be very pertinent to the question of the frequency of mitoses; namely, a periodicity[1] with regard to

FIG. 9. Resting oögonium with a definite chromatin reticulum; no "Nebendotter" present.

FIG. 10. Oögonium; very early prophase, spireme formation beginning.

FIGS. 9–17. From cells stained with Kernschwarz and Lichtgrün.

the divisions. It is well known that physiological factors may govern the time of mitotic divisions. A case in point is that recently described by Beckwith, previously mentioned; likewise, in certain insects and in many plants mitosis occurs at night only. The fact that many nuclei from one lot are in the same stage of division indicates, I believe, the effect of some physiological factor. What that factor is, I can only conjecture. Perhaps mitotic periods may occur only after a more or less prolonged fast on the part of the host, for then the energies of the parasite are not directed towards the assimilation of food.

During the maturation period the regular course is followed.

[1] This expression is not intended to imply that a definite amount of time intervenes between successive periods; they may recur at irregular intervals depending on some physiological factor.

Figs. 13 to 17 illustrate the process, but no attempt is made to give even a meagre outline of the behavior of the chromosomes in this period. However, attention is called to the appearance of the spindle. While spindle formation is quite regular, the achromatic fibres do not stain well and frequently the entire structure is overlooked. This fact taken with the smallness of the chromosomes and the prominence of the large cytoplasmic granules may well serve to veil the process of mitosis. In many cases one does not, at first, distinguish between the chromosomes and the

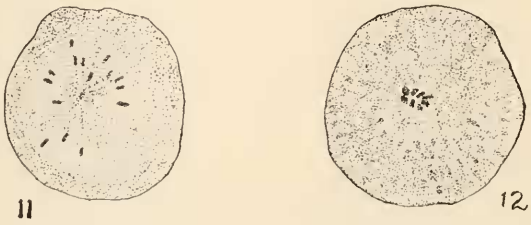


FIG. 11. Oögonium; polar view of an anaphase. This figure and the next are from early generations of oögonia.

FIG. 12. Oögonium; polar view of a telophase.

cytoplasmic granules, so nearly alike may they appear. While Fig. 15 shows clearly its mitotic character, the cell from which it was drawn was overlooked for a long time.

Fig. 17 is a case which suggests an "endogenous" division. According to Child, an endogenous division is that of a nucleus into two nuclei within the old nuclear membrane. Upon a superficial inspection the cell in question seemed to be in process of such division as shown by the outline in Fig. 18. Careful study, however, revealed the mitotic nature of the division. It is a late telophase with the chromosomes disintegrating; remnants of asters may be seen, as can a Zwischenkörper.

The appearance of this cell suggests the question of the relation between nuclear and cell division. Botanists have recognized the distinction between the two processes much more generally than have zoölogists. The latter have been accustomed to regard nuclear division as a sign of immediate cell division. Very often this is not the true state of affairs, for nuclear division may never be followed by cell division (Marshall), or a considerable period of time may elapse before a cell plate is formed. Fig. 17 shows

no sign of cell division although mitosis is almost complete. This is not an infrequent occurrence; actual nuclear division and certainly cell division may lag well behind spindle and chromosome division. Herein lies a fruitful cause for misinterpretation. A nucleus in which chromosome division has been completed would give every appearance of direct division upon constriction and subsequent division. Two nuclei in a single cell which had not begun to form a cell plate would also be misleading.

No true case of amitosis has been observed in the egg cell formation of the *Tænias* upon which I have worked.

Somatic Tissues. — The somatic tissues of tape-worms inside the cuticle include muscle fibres, excretory and genital organs, and a primitive nervous system. Surrounding all of these organs and filling all interstices is a large mass of parenchyma. A detailed study of all of these structures has been made by Child for *Monieza*. He reports that many cases of amitotic division occur and that illustrations of this fact might be multipled indefinitely.

I have made no investigation of the method of cell division in the excretory or nervous systems of *Tænia*. There is no reason

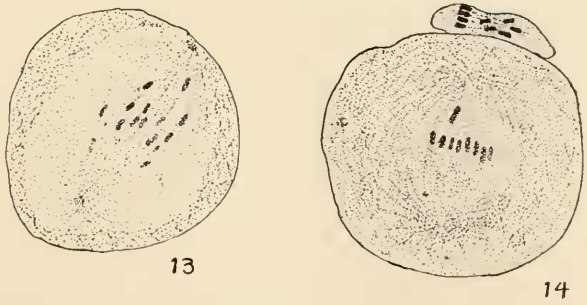
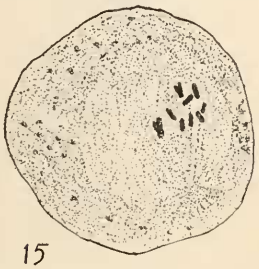


FIG. 13. First oöcyte; late metaphase.
FIG. 14. Second oöcyte; equatorial plate stage, first polar body dividing.

to believe, however, that the method of division in those organs differs from that of the genitalia or of the muscle fibers. We have in all of these systems cells that are specialized to a high degree. Even if amitosis be found here where rapid growth may be taking place that fact loses significance when the degree of specialization is considered. The various cell generations of a differentiating tissue differ from earlier generations only in a gain

in specialization and a loss in reproductive potentiality. Rapidity of division in these cases is a negligible factor. We are simply dealing with a special case under the old theory, if amitosis be found to obtain here. Amitosis, then, may well be expected in these systems.

The facts observed in the genitalia of *Tænia* do not bear out fully that expectation. The structure of the genital ducts and organs can be made out clearly and nuclei, cytoplasm, and intercellular substance seen. Yet cases of amitosis have not been demonstrated. On the other hand, a mitotically dividing nucleus is found only rarely. This may mean that mitosis is of very



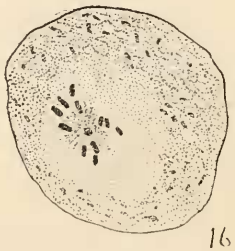


FIG. 15. Second oöcyte; early anaphase, spindle stained very lightly. Figs. 15–17 show the large cytoplasmic granules.

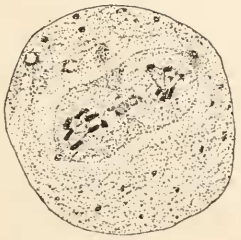
FIG. 16. Second oöcyte; telophase.

short duration, or more probably, that it is of short duration and occurs in waves; or, again, it may indicate that the nuclei divide amitotically. But whichever interpetation we may accept we do no violence to the theory of Ziegler and vom Rath.

The muscle-cells also furnish only negative evidence of amitosis. They are large, spindle-shaped cells from which contractile fibers extend. The cytoplasm is densely reticulated, rarely exhibiting the vacuolated structure described by Child (8). The quantitative relations of cells and fibers at different periods of development are of interest. Relatively more muscle cells are present in a young proglottid than in an old one, but the muscle fibers are much more developed in the later stages. The significance of this relation, which agrees closely with a similar parenchymal relation, will be discussed later. While satisfactory evidence as to the usual method of cell division has not been obtained, the observations on the material at hand favor mitosis as typical.

The meaning of amitosis in the parenchyma is of quite different import from that in specialized tissues. Parenchyma is a tissue from which others are derived; it is neither highly specialized, degenerating or pathological. The occurrence of amitosis here, therefore, would not be in line with the old theory.

Concerning the nature of parenchyma there has been much



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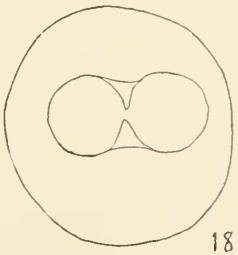

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FIG. 17. Second oöcyte in which mitosis is nearly complete, yet no sign of cell division. The daughter nuclei are being constricted apart and the chromatin masses are disintegrating. Zwischenkörper present.

FIG. 18. Outline drawing of Fig. 17. It gives the appearance of an "endogenous" division by amitosis. Fig. 17 shows that appearance to be entirely superficial.

controversy, but it is beyond the scope of this paper to enter into a discussion of the question or of the literature regarding it. In connection with an account of the method of cell division, however, a few observations concerning it cannot be avoided. The older view held by Leuckart and his followers was that the parenchyma develops from rounded and polyhedral cells, the latter sending out processes which interlace about the former. Moniez and his followers offered an opposed view. Opinion has by no means become settled even now. Child speaks "of the parenchyma cells if the syncytium which composes the parenchyma can properly be said to be composed of cells."

Parenchyma consists of calcareous bodies and irregularly shaped cells lying in a mass of material which seems to be intercellular. Of these, the first do not enter into the question under consideration, and the intercellular matter only figures as a ground substance in which the cells are imbedded. The cells vary in shape from elliptical and spindle-shaped to an irregular form with many protoplasmic processes. In size there is also much variation, due, largely, to the variation in the amount of the

cytoplasm, for the nuclei do not exhibit any such striking quantitative differences as does the cytoplasm. The cells do not seem to form a syncytium of which the ground substance is a part, as some writers have stated. The evidence seems to me to indicate that the ground substance bears a relation to the parenchyma cells similar to that borne by the intercellular matter of connective tissue, for example, to the connective-tissue corpuscle. That the parenchyma cells have definite boundaries is brought out clearly in the Lichtgrün preparations. I have seen no evidence for thinking the ground substance continuous with the cytoplasm. The nuclei of the cells show uniformity of structure as well as of size; they have a chromatin reticulum and usually a nucleolus. The parenchymal cell is thus seen to be a definite structure with a typical nucleus and a varying amount of cytoplasm.

Fig. 19 is a resting parenchyma cell of characteristic appearance. The cytoplasm is drawn out into strands upon the number

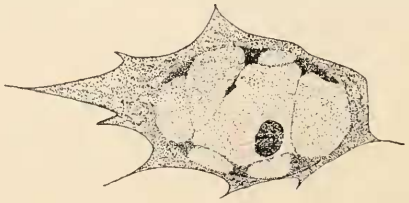

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FIG. 19. A typical parenchyma cell in the resting condition. This cell is from a proglottid in which the sex organs are only partially developed.

and size of which depends the width of the cytoplasmic band about the nucleus. Parenchyma shows regional differentiation in the relative amount of ground substance and cells and in the modification of the cytoplasmic parts of the cells only. In the younger cells the cytoplasmic strands are less numerous and frequently extend only a short distance. In older regions more cytoplasm is drawn out into the strands, leaving but a thin layer about the nucleus.

Professor Child has assumed through all his work that the absence of mitotic figures in tissues known to be growing rapidly is evidence of the occurrence of division by amitosis. This assumption is, of course, based on good *a priori* reasoning, but I

do not believe that it is borne out entirely by the facts. In *Tænia* the parenchymal cells are relatively less in number in the old tissue than in the younger, a relation which is only partially explained by the fact that other kinds of cells develop from the parenchyma. On the other hand, the ground substance in the young proglottid is more spongy and less in amount than in the older portions of the animal. These observations show that the growth of the parenchyma is not due alone to cell multiplication but also to the formation of new intercellular matter and to the greater development of the cytoplasmic strands. The growth of the cestode soma is due chiefly to the growth of the parenchyma and of the muscle cells and fibers. Other tissues, except the sex products, are practically negligible in accounting for increase in size. With regard to the growth of the muscle fibers a similar condition obtains in them as in the parenchyma, as is shown above. Thus we find growth depending not so much on the increase in the number of cells as in the increase in amount of products of cellular activity, that is, in fibers and intercellular material. Therefore, the rapidity of growth in the cestode body does not necessarily postulate a large number of dividing cells.

As to the method of cell division little can be said. No traces of amitosis appear. Cases of several nuclear divisions in a common cytoplasmic mass such as are figured by Child (11) for *Monieza* are not to be seen in *Tænia*, nor is any explanation of them afforded by the latter genus. I have also observed no cases of mitosis. The evidence at hand, I believe, does not warrant any conclusion as to the method of cell division in the parenchyma of this form.

Summary.

1. Amitosis has not been found to occur in the oögenesis of the cestodes studied.

2. All observations on the process of oögenesis point to mitosis as the usual method of cell division.

3. The presence of a "Nebendotter" which has peculiar staining properties gives a misleading appearance of amitosis.

4. Maturation is of the typical form.

5. Indirect evidence strongly suggests that physiological factors influence the frequency of mitotic divisions.

6. Nuclear divisions are not always followed immediately by cell division.

7. Only negative evidence as to the method of division in somatic structures has been found; there is no satisfactory evidence of amitosis and mitosis is not abundant.

8. Rapidity of growth in the somatic tissues of the cestodes body does not necessarily postulate many division figures.

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BIOLOGICAL BULLETIN

SOME EGG-LAYING HABITS OF AMPHITRITE ORNATA VERRILL.

JOHN W. SCOTT.

Several years ago while engaged in working out the development of the unfertilized eggs of *Amphitrite* I had occasion to collect large numbers of these worms. Mead, who worked on the cell-lineage of this annelid, had stated that nothing was known of their breeding season but that ripe sexual products could be had at irregular intervals during June, July and August. Collecting therefore during 1902 and 1903 was entirely at random. Incidentally the opportunity came to observe something about their egg-laying habits. During 1907 and 1908 I have used eggs of the same species for the study of other problems and have had a chance to further verify and extend the observations previously made. I wish here to express my appreciation to the directors of the Marine Biological Laboratory for their kindness and encouragement in helping me to prosecute this work.

The observations mentioned in this paper pertain to two questions in particular. I have always experienced considerable difficulty in obtaining, when wanted, mature sexual products of *Amphitrite*. The first question therefore deals with the time of egg-laying, in the hope that future investigators on this species may be saved some trouble and disappointment. The second question is concerned with the manner of depositing eggs. The eggs and sperm float free in the body cavity and these products are usually in various stages of development. This is true even at the time when worms deposit the mature products in a manner apparently normal. How is it possible to retain the cœlomic corpuscles and the unripe eggs and deposit the ripe sexual products? In a number of instances I have observed the act of

depositing eggs or sperm in the laboratory and I studied especially the manner of depositing the eggs.

My results in general are as follows. First, the egg-laying reflex is closely associated with the time of spring tide, the height of the season occurring at the time of new or full moon, or within two days after these dates. The best results were obtained in July, *i. e.*, a larger percentage of mature worms may be collected during this month than during any other. In early summer the period of sexual activity tends to occur a day or so later than the time mentioned, while in late summer the period tends to be earlier by the same amount. In regard to the second question I may say that the ripe and unripe products are not kept separate in laying with absolute exactness. Though among the first few hundred eggs deposited it is hard to find even one that is immature, toward the close of any given period of oviposition the immature eggs form a considerable percentage of the total number. But how does the worm keep these eggs apart in the first part of the period? A full consideration of this question is given later.

In order to understand the discussion of the two questions concerned it will first be necessary to say something of the habitat of this form and the environment under which it lives. These worms live in U-shaped, rather tough, mud tubes that break easily in digging. At one of the openings of the tube, sometimes at both, there is a volcano-shaped mound of sand or earth. The two openings are ordinarily from ten to eighteen inches apart and the depth of the lowest part of the tube is about three fourths the distance between the openings. The worms were collected in six different localities in the vicinity of Woods Hole. In these localities at the time of spring tide extreme high water and extreme low water differ by two to three feet. The tubes are found most abundantly on sandy flats which may vary from fine sand to a rocky character; they are also occasionally formed on sandy mud flats where the tide produces little current. The vertical distribution of the tubes is also comparatively limited. At the time of extreme low water tubes are rarely found beyond a depth of twelve or fifteen inches, and very few are found more than twelve inches above this line.

Probably two thirds of the tubes are within six inches, in a vertical direction, of this low water line at spring tide. The worms are more abundant on flats which are somewhat protected from strong currents. When the tide is running and the water comparatively shallow, it is quite common to see the worms at one of the openings, apparently feeding, the mouth just below the pit of the "volcano," and the numerous tentacles extending out for several inches in radial directions. It was found impossible to observe the deposit of sexual products under natural conditions, and this careful description of their habitat is given in order that we may better interpret the results obtained in the laboratory.

I. Time of Egg-Laying.

Verrill in 1871–2 described the occurrence of an annelid in Vineyard Sound to which he gave the name *Amphitrite ornata* Verrill. Several years later he gave the credit for the original description to Joseph Leidy who in 1855 had described what appears to be the same species under the name of *Terebella ornata* Leidy. No mention is made of egg-laying habits in either of these papers. To Mead belongs the credit of recording the first observations of this kind. He writes: "The limits of the breeding season are unknown. Although about eight hundred worms were collected in lots of twenty or thirty between the first of June and the last of August, only seldom were ripe eggs and ripe spermatozoa obtained. It is useless to cut the worms open, for if the sexual products are mature, they will be discharged, usually at about six o'clock in the evening, more often on the day of capture, sometimes the next day." I found little difficulty in verifying most of these results.

Amphitrite must necessarily be collected in the day time and when the tides are low. They were collected in quantities, from twelve to seventy-five specimens in each lot, and it is estimated that about two thousand adult worms were examined in the course of the four seasons during which their habits were under consideration. When dug they were washed free from the mud tube and placed in a bucket of sea-water to be carried to the laboratory. At times the males and females were placed in separate

buckets, but this practice was not often followed, for placing the sexes together did not cause them to discharge sexual products. In the laboratory all worms were carefully washed and isolated in separate dishes of sea-water. Eggs rarely fertilize if removed by cutting open the body wall ; if any considerable number are mature they will later be discharged in an apparently normal manner. The quotation from Mead, concerning the scarcity of ripe eggs and ripe spermatozoa, gives an accurate idea of my own results in that particular. But the time of discharge appears to be not so definite as one might think if guided solely by his description. Table I.

TABLE I.

Time.	A. M.					P. M.								Total.
	9	10	11	12	1	2	3	4	5	6	7	8	9	
Females.....			1	1		2	2	3		3	3	1		16
Males.....							1	3		2	2	1		9
Total			1	1		2	3	6		5	5	2		25

Showing time of day when *Amphitrite* begin to deposit sexual products. In the earlier work no record was kept of the males, hence the small number given in this table.

gives little support to his statement in regard to the time of day at which the worms deposit their eggs. As will be observed twelve of these worms, nearly one half of the entire number, deposited their sexual products between 6 : 00 and 8 : 30 P. M. An almost equal number shed their products between 2 : 00 and 5 : 00 o'clock, and two specimens were ovipositing between 11 : 00 A. M. and 1 : 00 P. M. Injury will sometimes cause *Amphitrite* to throw off their eggs, and of course it is possible that these two worms were injured in some way. However another fact must not be overlooked. It was noticed that nearly all worms deposited products from three to five hours after the low tide at which they were collected. This fact is undoubtedly important and probably explains some of the discrepancies found in the table. In a few instances collections were made between 5 : 00 and 8 : 00 A. M. ; more frequently they were brought to the laboratory about noon ; but, owing to local conditions, by far the largest number of worms was collected when the low tide occurred in

the afternoon. I believe this tendency to deposit eggs in the afternoon or early in the evening may be accounted for in the following way. When low tide occurs near mid-day or early afternoon, the sand flats are more exposed and reach a higher temperature than under ordinary circumstances. Metabolic changes are undoubtedly more active at these times and for a few hours immediately following. As a consequence, if the worms behave in their tubes as they do in the first few hours in the laboratory, the eggs are laid on a rising tide about the time of slack water. This time would be favorable for fertilization on account of the absence of strong currents. Some five hours later, the young blastula is ready to swim, as shown in a previous paper ('06).

TABLE II.

	Days Before.							Spring Tide.	Days After.							Total.
	7	6	5	4	3	2	1		1	2	3	4	5	6	7	
Females						2	1	6	2	2	1		1		1	16
Males						1	1	4	2	1	1					10
Females (injured).....								2	1				1	1		5
Males (injured)						1		2	1		1		1		1	7
Total.....	0	0	0	0	0	4	2	14	6	3	3	0	3	1	2	38
Number of times collected.....	3	4	1	5	4	5	3	7	4	5	6	6	3	3	6	65

Shows time at which eggs and sperm were deposited in reference to spring tide. Also the number of collections made in reference to the same period.

In all sixty-five experiments are here recorded, scattered pretty evenly throughout the summer from June 24 to August 23. In 1902 I was working on the unfertilized egg and few attempts were made at fertilization; hence the data are rather incomplete for that year. At first glance my data do not appear very significant, but one readily notices a general tendency for the oviposition to occur near the time of spring tides. This is especially true in those experiments where I found mature sexual products in greatest abundance. For example, in 1902 the best success in fertilization occurred on July 7, two days after new moon; in 1903 the best results obtained for the season were at the time of

new moon and the day following, July 23 and 24; in 1907 best results happened on July 26, two days after full moon; and in 1908 at new moon, July 28. The full meaning of these facts, however, is best brought out in Table II. Here I have shown the day on which eggs and sperm were deposited in the laboratory in reference to spring tide. In all I have recorded 38 instances where *Amphitrite* have shed eggs or sperm in an apparently normal manner. Twelve of these were known to be injured, and consequently their data are unreliable. But of the twenty-six uninjured worms twenty-three (88 per cent.) shed their products on or within two days of spring tide; twenty-four worms (92 per cent.) shed products within three days of spring tide; only one worm deposited eggs five days, another seven days, after the period named. Of the injured worms nearly sixty per cent. deposited products within two days of this period, while thirty-three per cent. missed the spring tide by more than four days. This suggests the possibility that, of those worms given as uninjured, perhaps the last two were injured internally. Again, the table shows that more worms laid eggs or sperm on the day of spring tide than on any other day; that the next largest number deposited products on the day following; that practically all sexual products are deposited within three days of spring tide with a tendency to follow rather than to precede this period. That the distribution of these figures is not due to a like distribution of the number of times worms were collected, is shown clearly by comparing them with the figures in the last line of the table.

In order to make a further test of the hypothesis that ripe sexual products are deposited most abundantly at the time of spring tide, on July 17, 1909, the date of the new moon, twenty adult *Amphitrite* were collected. Of this number twenty-five per cent., three females and two males, deposited ripe products, thus giving excellent confirmation to the hypothesis. The question arises how may we account for the periodicity in the time of egg-laying. Observations that have been made upon some other forms will help in our explanation.

Mayer ('02) has described the interesting case of the breeding habits of the Atlantic palolo. This worm "swarms at the surface before sunrise within three days of the day of the last

quarter of the moon between June 29 and July 28." "All eggs mature simultaneously at the time of the normal swarm." Later observations ('09) show that the time of swarming is not so definite as at first supposed. "When the last quarter of the moon falls late in July there may be a response to the first quarter as well as to the last quarter." "A dense swarm occurred on July 10, 1908, a fairly dense swarm on July 19." This behavior shows that a particular change of the moon has no direct effect on the time of swarming, as the moon was in first quarter July 6 and in last quarter July 20. Mayer also performed an important experiment to test the effect of tides on the time of swarming. He writes as follows: "Some worms swarmed normally on July 19 out of the rocks which had been maintained in a floating (tideless) live car for the six weeks previous to the swarm." This experiment appears to demonstrate that tides are without direct effect in producing the swarm. But, as Mayer concludes, it "may indicate that the changing pressure due to rise and fall of tide over the reefs is a contributory but *not a necessary* component of the stimulus which calls forth the breeding swarm."

However, it is known that the tide may form a sort of habit in the action of some animals. Gamble and Keeble ('06) have described a small, green, sedentary turbellarian, *Convoluta roscoffensis*, that occurs on the coast of Brittany. "It exhibits a periodic vertical movement whose rhythm is that of the tide." When the tide is out, they come to the surface, forming green patches on the sand. When the tide comes in they retreat below the surface into safer quarters. The remarkable fact is that when removed from the effects of tidal action by being kept in an aquarium, *Convoluta* continues to perform rhythmic movements in time with the tide. "The rhythm is not profoundly impressed upon it; after a day the movements of the patch in the vessel cease to synchronize with those in the open." This illustration suffices to show how an action may arise in relation to the tide without depending directly upon it.

In *Amphitrite* it has been observed that feeding is more continuous and more active as the time of spring tide approaches. At such times the great mass of tentacles radiate from one

opening of the tube and actively explore the vicinity for a distance of several inches. Their food consists of small bits of organic matter carried to the mouth through the action of cilia. One result of the feeding activity is seen in the fact that the immature eggs or sperm grow and mature very rapidly during the last few days immediately preceding the sexual period. Undoubtedly, this increased activity of the organism is stimulated by the higher temperature of the sand flats at the time of spring tide. The alternate increase and decrease in the depth of the water, thus altering the pressure, probably has some influence and the food supply at this period is certainly more abundant. We must conclude therefore that *the influence of the tides or moon is entirely secondary. The sexual activity in these worms is closely associated with a similar rhythmical period of greater bodily activity; and this greater bodily vigor of the animal is induced by conditions that depend upon the tides.* Furthermore, the periodic sexual reflex has acquired a sort of physiological basis in the organism, for the worms deposit sexual products normally, when removed from the influence of the tide. Still this reflex has not become a habit in the animal, at least not a strong one, for if a worm does not deposit its products within a few hours after being captured it rarely does so, and then not later than the following day.

II. Method of Egg-Laying.

The eggs of *Amphitrite* break loose from the matrix of the germinal epithelium in early stages of development and complete their growth while floating free in the cœlomic fluid. In a single worm they are usually found in the various stages of development. When first collected, all worms go through with a series of rhythmic movements of the body. When performed in the tube, these movements are evidently for the purpose of aeration, and they are kept up for some hours after the animals are removed from the tube, gradually diminishing in intensity. Each series of movements begins as a contraction near the posterior end of the body and travels forward; a second contraction follows, and frequently a third has begun before the first has disappeared. Between the contractions are wave-like enlarge-

ments that serve as moving valves to pump the water slowly through the tube. This pumping action may be demonstrated by placing a recently captured, uninjured worm in a U-shaped glass tube of suitable size. When *Amphitrite* is about to deposit its eggs the movements become more rapid and frequently more violent than usual. Quoting from my notes, the further process is somewhat as follows : " While the worm's body is undergoing the series of slow peristaltic movements, consisting of contraction-waves that begin near the posterior end and travel forward, the eggs ooze out in string-like, sticky masses that are soon scattered by the movements of the body, or by currents of water. The eggs are extruded through five openings in the anterior region of the body, the first opening being on the second segment back of the third pair of gills, or the sixth body segment, not counting the prestomium. Sperm is extruded through similar openings of the same number and location." These openings are nephridiopores that have become specialized as gonaducts, and it should be added that they are laterally placed, lying between the dorsal and ventral chætigerous lobes.

In a previous paper I have mentioned the fact that the eggs are greatly flattened in the polar diameter at the time of extrusion, and that the first polar spindle is in the metaphase. I have also mentioned above the fact that these worms possess the power of separating ripe and unripe eggs in the process of oviposition. How is this accomplished? Some dissections were made in an attempt to answer the question. It was found that this species possesses the same general arrangement of internal organs as that of other *Amphitrite* described by Meyer ('87). "Alle Nephridien der Terebelloiden, sowohl die vorderen als die hinteren, münden im Bereiche desjenigen Korperzonites, welchem sie angehören, einzeln und unabhangig von einander nach aussen ; ihre Wimpertrichter haben eine intersegmental Lage und öffen sich stets in das nächst vorangehende Segment." All the septa in the Terebellidæ are incomplete with one exception ; this one, the diaphragm, is strongly developed and separates the anterior region of the body cavity from the rest. In the species here described the diaphragm is between the fourth and fifth body segments. The external openings of the excretory nephridia open

on the third, fourth and fifth segments, but the inner openings are all anterior to the diaphragm. The inner openings of the post-diaphragmatic nephridia are fimbriated membranes, consisting of dorsal and ventral portions in close apposition, covered by cilia; each of these openings leads into a large membranous sac that is well supplied with blood capillaries and is also ciliated. These nephridia serve as gonaducts, and probably serve also as excretory organs. When egg-laying was first observed, I thought the collapse of the egg in the polar diameter might have a direct relation to the separating process, but there is nothing in the structure of the nephridia to indicate that the eggs undergo a sifting process. Besides the cœlomic corpuscles do not escape and they are smaller than either ripe or unripe eggs.

Somewhat similar phenomena have been observed by Gerould ('06) in *Phascolosoma*. "A few hours before egg-laying occurs, the nephridia become distended with a transparent fluid." "Ova that are ready for maturation, having the spindle of the first polar body in metaphase, are swept from the cœlom into the nephridia by the action of cilia which give rise to strong currents within the nephridium, setting from the nephrostome backward towards its posterior extremity. This is a most interesting process in that both the immature ovocytes, which are present in great numbers in the cœlomic fluid, and the cœlomic corpuscles are excluded from the nephridium, while the fully grown ovocytes are collected there in great numbers." Gerould presents a tentative explanation somewhat as follows. The transparent fluid, he thinks, is sea-water, taken in through the nephridiopore, and he believes that ova in the early stages of maturation probably absorb water while within the nephridium. "If eggs in the earliest stages of maturation show a tendency within the nephridium to absorb sea-water, may it not be assumed that ova at that stage are positively hydrotropic? On this supposition we may explain why such eggs are caught up from the cœlomic currents into the nephrostomal region, and thence carried into the nephridium." This assumption, however, is not to be seriously regarded and is, I believe, incorrect; at least it is incorrect in the case of *Amphitrite*.

It is rather an easy matter to separate the ripe eggs from the

immature ones and from the cœlomic corpuscles by decanting after stirring the mixture in sea-water; the largest ova and the ripe eggs, those with the first maturation spindle in metaphase, always settle more quickly to the bottom of the dish. The immature eggs then settle, and last of all the cœlomic corpuscles. *It is not an assumption, therefore, to say that the largest ova including those in the early stages of maturation, are more quickly influenced by gravity than the other bodies floating in the cœlomic fluid.* This is the important fact, and is no doubt due to the larger amount of yolk in such eggs.[1]

If, as Gerould supposes, a hydrotropic attraction is necessary to separate these eggs from the other bodies in the cœlomic fluid, then this influence would be useless for the purpose of separation when the contents of the body cavity are emptied in sea-water. For, surrounded by water, the hydrotropic influence would act in all directions and result in equilibrium. Such is not the case. Indeed, Gerould noticed such facts and in appendix B describes how large individuals should be opened in sea-water. "When the female with an abundance of eggs is found, the maturer ovocytes should be allowed to settle to the bottom, whereas the smaller ovocytes and cœlomic corpuscles should be decanted after a few seconds, and before they have had time to sink."

That gravity forms the differential by which the separation takes place is supported by a considerable number of facts. In the course of one period of oviposition, usually extending from one to one and one half hours, the eggs at first deposited are practically all in the metaphase of the first polar spindle; in the latter part of the period there is always a considerable number, and sometimes a majority, of ova deposited with the germinal vesicle intact. Upon killing a worm that is through egg-laying, one may still find a few scattered eggs in early maturation. If the separation depended upon a tropism, one does not see why it should be so much more complete at one time than another.

[1] Whether this tendency of large ova to settle quickly is due to a greater specific gravity, or to a greater mass in proportion to the amount of surface offering resistance, the end result is the same. This question must be decided by further investigation. In this paper we shall speak of the large ova as though they had a greater specific gravity than the smaller cœlomic bodies.

Again, the nephridia are not extraordinarily large, and each must be refilled many times in the course of one oviposition where thousands of eggs are deposited. Nor do the nephridia ever seem to be entirely filled with eggs; at least such is the case in worms that are killed almost instantly with hot sublimate acetic while in the act of oviposition. The meaning of this will appear in the explanation. It is also manifestly clear that the movements of the body referred to before, cause the body contents to move forward toward the diaphragm. As a rule, each effective contraction-wave stops a short distance posterior to the nephridia and holds for a moment the compressed cœlomic contents in the much distended anterior portion of the body. During the final part of a contraction-wave, a stream of eggs oozes out a short distance from each nephridiopore posterior to the diaphragm. The contraction movements, therefore, are necessary for the expulsion of the eggs.

The explanation which I believe accords best with all the known facts is as follows: First, it should be remembered that the nephridial sacs always occupy a lower position with reference to gravity than the nephrostome, whether the worm lies in a horizontal or vertical direction. We may then think of the nephridia as settling basins in which the heavier products are drawn off from the bottom after a certain amount has accumulated. Ciliary action undoubtedly would prevent lighter objects, such as the cœlomic corpuscles and the unripe eggs from settling in the basin; and when a sufficient quantity of the ripe eggs (heavier objects) have accumulated, pressure from the strong contraction-wave forces them through the nephridiopore. The separating process probably takes place during relaxation, between contraction waves. Owing to the opacity of the worm's body, it is impossible to actually observe this process, but all of the facts point to this simple explanation.

Summary.

1. In *Amphritite ornata* Verrill, the egg-laying reflex is closely associated with the time of spring tide; the height of any given period of egg-laying always occurs within two days of the time of new or full moon. Periods of oviposition occur in June, July and August.

2. The moon does not have any direct influence in producing the period of sexual activity. It is probable that the tide also has little, if any, direct effect on the process.

3. At spring tide, the worms feed more actively, the food supply is more abundant and the sand flats have a higher temperature. As this period approaches we also find a more rapid growth and development of immature eggs and sperm. Therefore, the period of sexual activity is closely associated with a synchronous period of greater bodily activity, and this greater vigor of the animal is induced by conditions that depend upon the tide. In this way we may explain how oviposition in *Amphritite* has become a sort of reflex habit associated with the time of spring tide.

4. When a worm is sexually mature, the cœlomic fluid contains cœlomic corpuscles and eggs in various stages of development. At oviposition the worm extrudes ripe eggs, and toward the end of the process some of the immature ones, but always retains the much smaller cœlomic corpuscles.

5. Since the mature eggs sink faster in sea-water than the smaller immature ones, and all eggs sink faster than the cœlomic corpuscles, it is believed that the larger eggs have a greater density than the other bodies in the cœlomic fluid; and it is entirely probable that the apparent selection of ripe eggs and the rejection of immature ones is due to the different effects produced by nephridial currents upon bodies of apparently different densities.

6. The position of the nephridial sacs, and the arrangement of cilia on the nephrostomes and within the sacs, is such that we may regard the nephridia as a set of settling basins in which the separation takes place. Contractions of the worm's body then aid in expelling the ripe eggs from the nephridial sacs.

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ON THE USE OF MAGNESIUM IN STUPEFYING MARINE ANIMALS.

ALFRED G. MAYER.

It is well known that Tullberg, 1892, discovered that an excess of magnesium added to sea-water causes anesthesia in marine animals, thus permitting them to be killed in an expanded state.

During the course of some physiological experiments carried out at the Marine Laboratory of the Carnegie Institution of Washington at Tortugas, Florida, I found that marine animals can be anesthetized much more rapidly and completely than by Tullberg's method if we simply place them in a pure aqueous solution of $MgSO_4$ or $MgCl_2$ of three eighths molecular concentration. They then subside into complete relaxation without initial stimulation, and after remaining for an hour or two in the solution they may be killed in any manner whatsoever without becoming distorted through contraction. Some distortion is often produced in Tullberg's process, due to the calcium and sodium of the sea-water, but in a pure aqueous solution of magnesium the relaxation of the muscles is complete. This method has been tried upon scyphomedusæ, ctenophoræ, actinians, annelids, nemertians, phascolosoma, and nudibranchs with marked success, and appears to be especially suitable for the stupefying of highly sensitive and contractile marine animals which become hopelessly distorted if killed by ordinary methods.

It is interesting to observe however that while magnesium is the most potent anesthetic for the neuro-muscular system it is the most powerful stimulant among the ions of sea-water or of blood-salts for the movement of cilia. Indeed I find that the ions of Na, Mg, K and Ca affect cilia in a manner the exact *opposite* of their effect upon muscles and nerves. Thus Na is the most powerful neuro-muscular stimulant, and the most pronounced inhibitor for the movement of cilia. Mg is the greatest inhibitor for nerves and muscles and the strongest stimulant for the movement of cilia. A weak concentration of K at first excites and then depresses the

neuro-muscular system, and at first subdues and afterwards stimulates the movement of cilia. Ca is a depressant for nerves and muscles but a weak stimulant for cilia. NH_4Cl is a primary stimulant for muscles but soon produces depression, while upon cilia its effect is the reverse, a primary cessation of movement being followed by recovery. The CO_2 ion inhibits muscular activity, while in weak concentration it produces a primary depression of cilia followed by a recovery of movement.

REGENERATION IN FUNDULUS AND ITS RELATION TO THE SIZE OF THE FISH.

G. G. SCOTT.

Przibram ('09) in his treatise on regeneration has reviewed the work bearing on the relation between the age of the animal and the ability to regenerate. The general conclusion is in the form of a law — "Die Regenerationsfahigkeit nimmt mit zunehmenden Alter eines Tierexemplares ab —." In the BIOLOGICAL BULLETIN ('07) the author came to the conclusion that the regeneration of the caudal fin of *Fundulus heteroclitus* was greater in the shorter than in the fishes of medium length and greater in these than in the longest fishes.

That conclusion was reached by comparing the average specific regeneration of the various groups. The specific regeneration is obtained by dividing the actual amount regenerated by the length of the animal, giving as a result the percentage amount regenerated by that specimen. This term was introduced by Zeleny. Now if we take any number and divide it successively by a series of numbers each greater than the preceding we will obtain a series of quotients each successively smaller and smaller. On referring to the experiments in his former paper the author found that the actual amounts of regeneration differed but little in the various specimens used, while the length of the animals increased, so that the case is as stated above — *i. e.*, that the greater specific regeneration in the shorter fishes is due largely to the fact that the divisor in case of short body length is smaller than the divisor in case of long body length, while the dividend (the actual amount regenerated) is about the same, so that naturally we should get smaller and smaller quotients, which in this case would be specific regeneration. This is seen when we refer to the former paper.

To test the matter further the writer repeated the experiment during the summer of 1908 using a larger number of fishes. The caudal fin of each specimen was removed at the same relative level on August 4 and the fish were placed in running sea-

TABLE I.

Fundulus heteroclitus.

	Length.	Amount Regenerated.	Specific Regeneration.		Length.	Amount Regenerated.	Specific Regeneration.
1	4.57 cm.	.57 cm.	.1247	55	6.80 cm.	.70 cm.	.1029
2	4.68	.54	.1154	56	6.81	.53	.0778
3	4.79	.58	.1210	57	6.86	.64	.0933
4	4.87	.66	.1355	58	6.89	.59	.0856
5	4.96	.65	.1311	59	6.90	.65	.0942
6	4.97	.60	.1201	60	6.94	.59	.0850
7	5.10	.54	.1058	61	6.96	.64	.0919
8	5.12	.59	.1152	62	6.97	.66	.0946
9	5.16	.58	.1124	63	7.10	.62	.0873
10	5.17	.54	.1044	64	7.12	.52	.0730
11	5.28	.60	.1117	65	7.24	.68	.0939
12	5.30	.61	.1151	66	7.26	.80	.1102
13	5.38	.64	.1187	67	7.29	.67	.0905
14	5.40	.60	.1111	68	7.29	.63	.0864
15	5.42	.54	.0996	69	7.38	.58	.0786
16	5.43	.64	.1178	70	7.39	.70	.0947
17	5.44	.61	.1122	71	7.40	.56	.0757
18	5.44	.53	.0974	72	7.44	.69	.0927
19	5.49	.65	.1184	73	7.45	.53	.0711
20	5.52	.69	.1250	74	7.48	.56	.0748
21	5.63	.66	.1172	75	7.49	.50	.0667
22	5.64	.61	.1081	76	7.50	.54	.0720
23	5.69	.59	.1031	77	7.50	.49	.0653
24	5.70	.78	.1368	78	7.52	.50	.0665
25	5.70	.66	.1158	79	7.53	.60	.0975
26	5.73	.68	.1193	80	7.56	.58	.0766
27	5.79	.60	.1036	81	7.60	.70	.0921
28	5.80	.58	.1000	82	7.62	.73	.0945
29	5.90	.60	.1002	83	7.69	.54	.0702
30	5.93	.65	.1096	84	7.74	.54	.0967
31	5.96	.57	.0956	85	7.77	.62	.0798
32	5.96	.64	.1074	86	7.87	.60	.0763
33	5.99	.78	.1302	87	7.88	.54	.0685
34	6.04	.67	.1109	88	7.92	.56	.0701
35	6.20	.62	.1000	89	7.94	.55	.0683
36	6.26	.67	.1070	90	8.00	.47	.0587
37	6.40	.70	.1094	91	8.00	.65	.0813
38	6.46	.50	.0774	92	8.07	.59	.0731
39	6.48	.68	.1049	93	8.10	.62	.0765
40	6.49	.52	.0801	94	8.23	.54	.0656
41	6.50	.60	.0923	95	8.23	.52	.0632
42	6.50	.60	.0908	96	8.25	.55	.0666
43	6.50	.57	.0877	97	8.27	.57	.0689
44	6.53	.53	.0812	98	8.27	.45	.0544
45	6.53	.54	.0827	99	8.37	.37	.0442
46	6.55	.63	.0962	100	8.38	.65	.0775
47	6.62	.47	.0710	101	8.38	.56	.0668
48	6.63	.60	.0815	102	8.39	.60	.0671
49	6.65	.63	.0947	103	8.40	.65	.0774
50	6.69	.63	.0942	104	8.56	.64	.0746
51	6.72	.54	.0803	105	8.69	.50	.0575
52	6.73	.63	.0937	106	8.91	.56	.0628
53	6.75	.42	.0622	107	9.00	.53	.0590
54	6.76	.64	.0974	108	9.73	.55	.0656

water in the Biological Laboratory of the U. S. Bureau of Fisheries at Woods Hole, Mass. I am indebted to Dr. Francis B. Sumner, the director of the laboratory, and to the commissioner, the Hon. George M. Bowers, for the facilities extended. The fishes were fed regularly until September 5, a period of a month, during which time new caudal fin tissue regenerated from the cut surface of each fin. The fishes were then removed, carefully preserved and later measured. There were alive, on September 5, 108 *Fundulus heteroclitus* and 50 *Fundulus majalis*. Table I. shows the length, the amount regenerated and the specific regeneration of each *Fundulus heteroclitus*. Following this is Table II. which shows the same with regard to the *Fundulus majalis* used.

TABLE II.

Fundulus majalis.

	Length.	Amount Regenerated.	Specific Regeneration.		Length.	Amount Regenerated.	Specific Renegeration.
1	6.79 cm.	.60 cm.	.0899	26	8.08 cm.	.47 cm.	.0582
2	6.95	.58	.0835	27	8.13	.39	.0480
3	7.13	.53	.0743	28	8.18	.60	.0733
4	7.32	.54	.0738	29	8.20	.50	.0549
5	7.40	.55	.0743	30	8.23	.40	.0485
6	7.40	.45	.0601	31	8.24	.63	.0764
7	7.45	.55	.0768	32	8.26	.49	.0593
8	7.49	.55	.0734	33	8.26	.52	.0629
9	7.49	.55	.0734	34	8.33	.53	.0636
10	7.50	.49	.0658	35	8.45	.49	.0580
11	7.50	.55	.0733	36	8.50	.50	.0419
12	7.55	.48	.0635	37	8.57	.53	.0618
13	7.55	.40	.0530	38	8.60	.50	.0580
14	7.60	.53	.0698	39	8.60	.42	.0488
15	7.61	.55	.0722	40	8.67	.44	.0588
16	7.67	.53	.0691	41	8.72	.57	.0654
17	7.77	.55	.0708	42	8.75	.39	.0466
18	7.80	.55	.0705	43	8.80	.38	.0432
19	7.84	.35	.0446	44	8.96	.40	.0451
20	7.88	.50	.0634	45	8.91	.46	.0516
21	7.89	.45	.0570	46	9.17	.50	.0545
22	7.93	.59	.0744	47	9.20	.60	.0652
23	7.93	.55	.0693	48	9.60	.30	.0318
24	7.95	.56	.0704	49	9.73	.42	.0432
25	8.00	.40	.0500	50	10.50	.49	.0466

On examining the specific regeneration in the case of *Fundulus heteroclitus* there is seen to be a gradual fall in percentage from the shorter to the longer fishes. To make this more plain we can divide the fishes into groups ranging from the shorter to the

longer, differing in length from the adjacent groups by one half centimeter, find the average specific regeneration of each group and express the results as follows :

Group.	Range in Length.	Number of Specimens.	Average Specific Regeneration.
1	4.5– 5.0 cm.	6	.1243
2	5.0 5.5	13	.1108
3	5.5 6.0	14	.1123
4	6.0 6.5	7	.0885
5	6.5 7.0	22	.0878
6	7.0 7.5	13	.0843
7	7.5 8.0	14	.0762
8	8.0 8.5	14	.0672
9	8.5 9.0	3	.0649
10	9.0 9.5	1	.0590
11	9.5 10.0	1	.0656

From this arrangement it would appear that the shortest regenerated 12 per cent. of their length while the longest regenerated 6 + per cent. and that on the whole as the length of the fishes increases the percentage regeneration decreases. On the other hand if we run over the column giving the actual amounts of regeneration we see that in a general way they are much the same for the longer as well as for the shorter fishes. This becomes evident when we ascertain the average actual regeneration for each of the groups mentioned above. This can be arranged as follows :

Group	1	2	3	4	5	6	7	8	9	10	11
Av. Reg., cm.	.60	.59	.65	.62	.59	.62	.58	.56	.57	.53	.55

On examining again the table giving the actual regeneration of each specimen we find some that may be regarded as extreme variants. These are nos. 24 and 33, in which the regeneration is .78 cm., no. 66 with a regeneration of .80 cm. and no. 99 in which the regeneration is .37 cm.

If we make allowance for these and obtain new averages for the groups in which they occur our series will be as follows. We will disregard the last two groups on account of the small numbers of specimens.

Group	1	2	3	4	5	6	7	8	9
No. of Sp.	6	13	12	7	22	12	14	13	3
Av. Reg., cm.	.60	.59	.63	.62	.60	.60	.58	.57	.57

If we compare the average regeneration of groups 1 and 2, representing 19 fishes from 4.5 cm. to 5.5 cm. in length, with

the average regeneration of groups 8 and 9, representing 16 fishes from 8.0 cm. to 9.0 cm. in length, we find that there is a difference of but .025 cm. Again, if we compare the average regeneration of groups 2 and 3, representing 25 short fishes, with that of groups 7 and 8, representing 27 longer fishes, we find a difference of but .035 cm. The relation of the specific regeneration to the actual regeneration is represented by the following diagram (Fig. 1) in which the base line represents the length of the fishes — the upper curve *A–B* was formed by joining the points representing the specific regeneration in the various groups and therefore represents the relation of the specific regeneration to length. The lower curve, *C–D*, was formed by drawing a line through the points representing the actual regeneration in the various groups and therefore represents the relation of the actual regeneration to length. It is to be noted that while on the whole the curve representing relative specific regeneration falls, at the same time the curve representing the actual regeneration remains almost parallel with the base line although it will also be noted that there is an indication of a slight decrease in regeneration. There is a strong indication, however, that the longer fishes have regenerated almost as much tissue as the shorter in the same length of time.

But what is the condition in the case of the *Fundulus majalis* the results of the experiment with which are given in Table II. On arranging the results in a way similar to that used in the case of *Fundulus heteroclitus* we have the following :

Group.	Range in Length.	Number of Specimens.	Amount Regenerated.	Average Specific Regeneration.
1	6.5– 7.0 cm.	2	.59 cm.	.0867
2	7.0 7.5	7	.52	.0709
3	7.5 8.0	15	.51	.0658
4	8.0 8.5	11	.49	.0594
5	8.5 9.0	10	.46	.0521
6	9.0 9.5	2	.55	.0598
7	9.5 10.0	2	.36	.0373
8	10.0 10.5	1	.49	.0466

Here again it will be observed that there is a gradual decrease in the specific regeneration. The number of specimens in groups 1, 6, 7 and 8 is so small that too much value must not be placed on them. Although the total number of specimens is smaller in

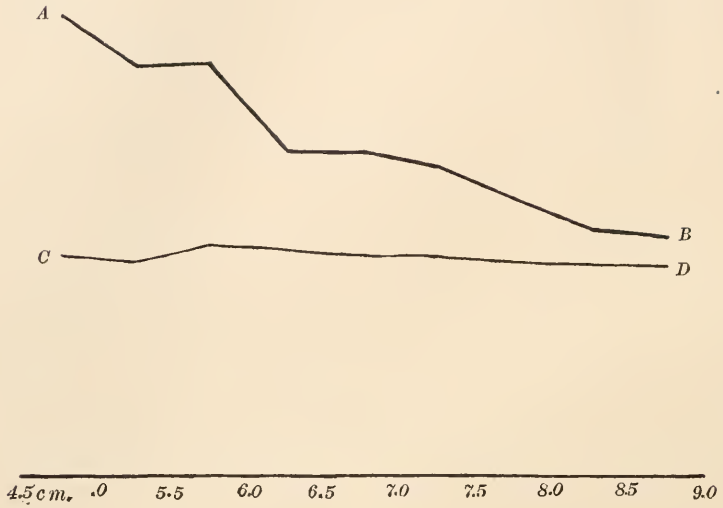

Fig. 1

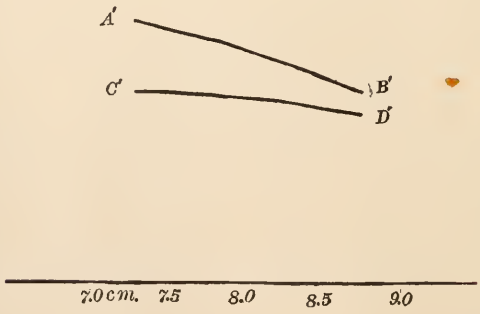

Fig. 2

this case, yet it is seen that the average regeneration is about the same, although here again is observed the slight decrease. The average regeneration of groups 2 and 3, consisting of 22 fishes between 7.0 and 8.0 cm. in length, is .515 cm., while that of groups 4 and 5, consisting of 21 fishes between 8.0 and 9.0 cm. in length, is .475 cm., showing a difference of .04 cm. between the two groups. These results are shown graphically in Fig. 2, in which the curve $A'-B'$ shows the relation of the specific regeneration to length, while the curve $C'-D'$ shows the relation of the actual amount regenerated to the length. Turning to the results recorded in the former paper we find that they are less satisfactory on account of the smaller numbers. But arranging the results recorded there in a manner similar to that used here we find that on the whole the longer fishes regenerate almost as much as the shorter, although the indication of the slight diminution of actual regeneration with age is not so clear, which may be due to the fact that there are less specimens in the various groups.

The general result seems to be then that the amount of regeneration in the period of time referred to appears to be about the same in fishes of all lengths, although there is an indication of a slight decrease in the case of increasing body length.

Professor Zeleny in a paper to be published shortly (Oct., '09) in the *Journal of Experimental Zoölogy* has found with respect to the regeneration of the tail of the salamander, *Amblystoma jeffersonianum* that rate of regeneration was as follows:

In a series	22.6	mm.	long	there was a regeneration of	.39	mm. per day.
"	26.5	"	"	"	.41	"
"	26.8	"	"	"	.39	"
"	51.6	"	"	"	.32	"
"	54.1	"	"	"	.27	"

These results agree with those described above. There is a maintenance of a high degree of regenerative power in the older specimens. There is also an indication of a slight decline such as we found in the case of the fishes used.

In carrying on an extensive series of observations on the age of fishes Fulton ('06) estimated the age by finding the different modal lengths that occurred in a large number of specimens of a given species. Though the numbers used in this experiment are

far too few to enable one to estimate the exact age of the various groups yet reasoning as Fulton did we can at least say that the longer fishes are the older. Stating the matter in terms of age the above experiments appear to indicate that within the limits of age as represented in the series the actual regeneration is the same or slightly decreases with age. It may be objected that the longer (or older) fishes regenerated more in mass than the smaller and that therefore should we determine the mass for each specimen we might find that the larger regenerated more than the smaller. To answer this objection let us suppose that Figs. 3 and 4 represent respectively the caudal fins of one of the shorter

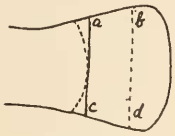

FIG. 3.

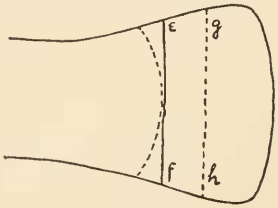

FIG. 4.

and one of the longer fishes. The straight vertical line in each case represents the place of amputation. The dotted vertical line represents the outer limit of new regenerating tissue at the end of a month. According to our results the perpendicular distance from the line *a–c* to the line *b–d* is nearly the same as that from *e–f* to *g–h*. When the amputation was made it left cells exposed along the surfaces represented by the lines *a–c* and *e–f*. In a short time the regeneration of new tissue began. In Wilson's "Cell," 00, page 388, we find that "measurements of cells from the epidermis, the kidney, the liver, the alimentary epithelium and other tissues show that they are on the whole as large in the dwarfs as in the giants. The body size depends on the total number of cells rather than on their size individually considered and the same appears to be the case in plants."

So we can conclude that the cells from which new tissue regenerates along the surface represented by the line *a–c* are of the same size as those represented by the line *e–f*. It is apparent that the tissue along the direction of *a–b* has been formed from cells at *a*, and that the tissue along the line *c–d* from cells at *c*.

So for every point in the line *a–c* the tissue opposite every point in this line has been formed from cells in the line *a–c* outward in a direction perpendicular to that line. And so for the newly formed tissue in case of Fig. 4, the tissue opposite every point in the line *e–f* has been formed from cells in the line *e–f* outward in a direction perpendicular to the line *e–f*. Of course in the case of larger fishes (Fig. 4) it is apparent that a greater mass of tissue is formed than is true of smaller fishes (Fig. 3). But is this not due to the fact that there are more cells along the surface represented by the line *e–f* from which regeneration can proceed than there are similar cells along the surface indicated by the line *a–c*. The solution of the problem as to the relation between age and rate of regeneration depends upon the results of measurements made in this manner. And by the amount of tissue regenerated has been and will be meant the length of newly formed tissue measured outward from the line of amputation.

If the cells in the shorter and longer (younger and older) fishes have the same degree of activity then the same amount of tissue ought to be formed outward in a line perpendicular to the cut surface in the same length of time. This is seen to be practically the condition in the case of the experiments presented here. What explanation can be offered for this? Jordan, '05, speaking of the growth of fishes says that "Most of them grow as long as they live and apparently live until they fall victims of some stronger species." Fulton, '06, gives the following law with regard to the growth of fishes: "Fishes approximately double their size and increase their weight about eight times after they have reached sexual maturity." Probably most of the fishes used in these experiments had reached sexual maturity. May we not then correlate this maintenance of a high degree of regenerative power with the continuous growth throughout the life of the members of this group.

But there appears to be a slight decrease in the amount regenerated as the age increases. In computing the difference of the means and the probable differences between adjacent groups of the different series it was found in most cases that at least twice the probable difference was less than the difference of the means and only in case of extreme groups was the difference of the

means less than three times the probable difference. This indicates that in adjacent groups the amount of regeneration is very nearly the same, but that on the whole there is a tendency toward a decrease, as we pass from the younger to the older. We can sum up our results in this statement: The power to regenerate new tissue remains remarkably active throughout life but as the fish grows older this power gradually diminishes, which after all is in agreement with Przibram's law. This also is in harmony with the view that regeneration is a growth phenomenon as shown above. Minot, '90, says: "There is a progressive loss of vitality going on probably throughout the entire period of life." Kellicott, '08, found that the organs of the dog-fish which have to do with nutrition and therefore the growth of the organism increase by constantly decreasing increments with increasing size of the animal. The slight decrease in regenerative power which we have noted above parallels then this slowly decreasing rate of growth characteristic of all animals, but which decrease is less evident in those forms having indeterminate growth such as fishes.

Kellicott, in the paper referred to above, found that while the organs of nutrition have increments of growth successively smaller, yet the "muscles and supporting tissues seem to outgrow the brain and viscera leading ultimately to a loss of physiological balance within the organism." But this decrease in growth of the organs of nutrition does not come on suddenly but gradually, so that it must eventually cause a gradual retardation of the growth of the entire animal. If this be true and if the rate of regeneration is affected by the rate of growth, then we should expect to find evidences of gradual diminution in the rate of regeneration. We have seen an indication of this in the above experiments. Finally, it has been noted that mammals and birds have little power of regeneration as compared with amphibia and fishes. May not this be possibly correlated with the different types of growth which these groups possess.

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SOME LIGHT REACTIONS OF THE MEDUSA GONIONEMUS.

L. MURBACH.

In the following notes it is my purpose to record some observations I have made on the behavior of *Gonionemus*[1] to light, after the experiments made a few summers ago on the reactions of its subumbral papillæ to light, and to include in the discussion points on which other observers are not agreed. I shall refer only to these publications.

After observing the behavior of *Gonionemus* for a considerable time the following brief statements will be found to hold concerning their habits. As darkness approaches the medusæ become restless in their native haunts where they are either lying inverted on the bottom, the apex of the bell being heavier, or clinging to plants and other submerged objects. Although all the tentacles in this species have adhesive pads near the free end, yet the animals attach by only a few, the remaining tentacles being spread out in all directions ready to catch their passing prey. When still daylight above the water it is becoming dusk, we may say, at their depth, and among the plants from one half to several meters deep. Within an hour after dark the eggs and sperm are deposited and their intermingling in the sea water increases many times their chance of development. Thus their locomotion in early evening is of great value as then more eggs will be fertilized. The dehiscence of the eggs and sperm has been shown to be due to the diminution or withdrawal of light, and seems rather direct evidence of an external stimulus causing a physiological change. For considerable time after the dehis-

[1] There would seem to be no need of stating that the Woods Hole species is the one under consideration. The experiments on which these notes are based were made at the Marine Biological Laboratory, at intervals between other work in the summer. I gladly acknowledge the courtesy of the Director in continuing to grant the necessary facilities. It will be found that some points differ from a report made on this subject in the winter before the Michigan Academy of Science (Annual Report, 1909) as I have been able to make additional experiments this season.

cence of the sex products the animals lie expanded on the bottom or suspended, and may remain so as long as the light conditions are the same, full darkness having set in. It is probable that much of their prey is captured at this time. As the light grows brighter above, they again move about until they get into weaker light which generally takes them into lower regions or into shaded places, in weak or subdued light. Whenever disturbed, especially by change of light intensity, this medusa swims about in all directions, stopping to float down with expanded tentacles and inverted bell. Again it displays a most striking behavior; it swims almost vertically to the surface of the water by pulsations of the muscular bell, turning over at the surface, expanding and gracefully floating down. Much has been made of this particular behavior by nearly all those who have observed *Gonionemus* for some time. It has been referred to as "fishing" and "surface reaction," the latter term being more satisfactory because less anthropomorphic. The main feature of this behavior is the swimming up toward the surface and floating passively down again after turning over at the surface. This may be renewed as long as the same stimulus acts, or until the condition of the medusa changes so that it no longer responds.

From the foregoing it will be readily understood that these medusæ are sensitive to light influences, getting away from strong light — especially during the earlier part of the day and afternoon — later again moving up toward the fading light. In general, Yerkes,[2] who has made most observations on the reactions of *Gonionemus*, says: "Clearly, the animals are attuned, so to speak, to a certain range of light intensity, and are negative in their reactions to higher intensities." Any marked change in this intensity causes locomotion which under natural conditions brings the medusa into the light suitable for its life processes. Whether this optimum intensity is constant or changes with the activities of the animal has not been determined, but ordinary observation indicates that its range is not very wide; it may be called weak light.

On account of the influence of light on these medusæ they collect in the weaker light of an ordinary glass aquarium placed

[2] *Amer. Jour. Physiol.*, 1903, Vol. IX., page 286.

before a window (cf. Fig. 1). If the light is still stronger than their normal they will continue in their attempt to get farther away. When the aquarium is evenly lighted with subdued light the medusæ are evenly distributed and generally at rest. This may be brought about by darkening the window, and the room if necessary, until the light is uniformly weak. If, now, the side

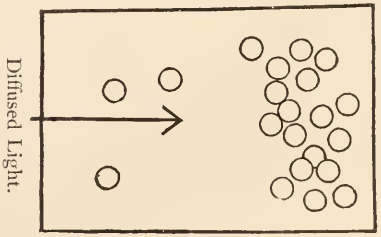

FIG. 1. Top View.

of the aquarium away from the window is darkened still more, then the medusæ are again set into activity, moving about until, after some minutes, they will be found collected in the side of the aquarium near the window.[3] In nature there is probably no collecting in groups, as they are not confined.

Directed Movements.

The main question in my mind has always been whether these medusæ are really directed by light, or properly oriented, even if they could keep the course, *i. e.*, continue oriented or directed. My own experiments have led me to believe that the movements of *Gonionemus* in response to light are not so definitely directed by light as has heretofore been held, and that quantitative differences near their optimum constitute the natural light stimulus. In regard to directed movement, Yerkes[4] says: "It is impossible because of the form of the medusa and its mode of locomotion, that the direction of its movements be as accurately determined by light stimulation as are those of . . . other animals whose structure permits of more accurate orientation in reference to the source of light." While no doubt in a measure true, to me it seems that this statement is not wholly borne out by the fact that it can swim

[3] Because of the observation above I cannot agree with Morse that *Gonionemus* is not positively phototactic.

[4] *Amer. Jour. Physiol.*, 1902, Vol. VI., p. 448.

in nearly straight lines when coming to the surface, in the so-called surface reaction. In fact, the only stimulus to which it seems to respond in pretty straight lines is gravity, though the question of the influence of light in the "surface reaction" is difficult to decide.[5] Yerkes's statement[6] "that the direction of its movement is definitely determined by light" is based on analogy and not on experiment. In this paper[7] such expressions as "movement toward the source of light," and "strong light . . . soon repels the animals"; again, "an animal passes from the shadow into the sunlight" (page 305), I do not take to mean that the animals swam directly toward or from the light. However, if this is meant, the strongest argument in favor of the medusæ's swimming in a direct line not vertical is (page 282) where a medusa inhibited by strong light, starting up again, "usually turns in such a way as to move back into the shaded region." Morse[8] reports a similar experiment differing in that he observed the movement of medusæ in the sunlit half of the dish, saying, "the medusæ begin to swim in all directions." With this my observations agree. Indeed, in Yerkes's answer to this criticism of Morse's, having repeated the experiment, he says: "I found that when the animals swam so far into the sunlit region before turning over that they were entirely in the sunlight when they came to rest on the bottom of the dish, they moved away from the region of shadow about as often as toward it."[9] In this, then, he agrees with Morse's contention that the medusæ do not turn directly toward the shadow and swim into it more often than away from it. In fact he adds (page 462) "with the light perpendicular to the bottom of the vessel I obtained the same results as Mr. Morse. There was no evidence of the directive influence of light."[10] But Yerkes (page 461) also points out that his infer-

[5] This "surface reaction" has been observed in three species of the genus, geographically far enough removed from each other that it seems to indicate an ancestral character.

[6] *Amer. Jour. Physiol.*, 1903, Vol. IX., page 285.

[7] *Amer. Jour. Physiol.*, 1903, Vol. IX., p. 284.

[8] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 454.

[9] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 461.

[10] Page 462 Yerkes suggests that the contradiction between himself and Morse in regard to oblique light might disappear if Morse's meaning of the term were more fully explained. Turning to Morse's experiment we find (pp. 453–454) that he used

ence from his original experiment was correct for all medusæ falling on the border between sunlight and shade so that "part of the body is in the shadow."

Does Light Orient *Gonionemus?*

Although *Gonionemus* does not move parallel to the direction of stronger light it has been held that its movements are directed by stronger light and that it thus gets from an unfavorable to a favorable light place by direct responses or movements suited to this purpose. Yerkes says:[11] "This is apparently accomplished by the more forceful and earlier contraction of that side of the bell farthest away from the shadow." In the case of other stimuli (tactual and electrical) he had demonstrated this but not in the case of light. Though, page 285, we read: "Observation indicates that the side of the organism which is exposed to the most intense light contracts first and most strongly thus forcing the bell over," yet there is scarcely the weight of proof in this observation. In a later paper Yerkes[12] says: ". . . brilliant illumination of one side of the bell . . . brings about movement toward the region of lower illumination." This is based on an experiment of throwing sunlight on part of a medusa. He gives 66 per cent. as turning toward the region of lower illumination.

Morse[13] restates the same explanation of the turning mechanism, and from the experiment of mutilating one side of the medusa shows that by the resulting one-sided contraction circle swimming is induced. In a later publication[14] he has shown that light has the same effect, *i. e.*, to turn the animal. Half of a medusa in the dark was illuminated by a vertical beam of light. This caused ". . . the medusa to swim vertically upward, and it was only after it had pulsated three or four times that its path veered from the perpendicular. The result of one hundred trials,

only sunlight falling perpendicularly, and in another experiment in which he used oblique light it had no reference to Yerkes's former experiment. More medusæ would fall in oblique light with bodies partly in the shadow than in vertical.

[11] *Amer. Jour. Physiol.*, 1903, Vol. IX., p. 284.

[12] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 461.

[13] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 451.

[14] *Amer. Nat.*, 1907, Vol. XLI., p. 683.

upon different individuals in the main" gave 70 per cent. in favor of the view "that light has a directly orienting effect." In considering this experiment we should know how many different individuals were used, and should bear in mind that the time limit for response was from five seconds to three minutes, and that the turning began to show only after several pulsations. This would seem to be different from Yerkes's view which is that the medusæ turn directly on stimulation by strong light.

As my experiments were made before the above results were published, and they favor the view of Yerkes that strong light turns the animal immediately they may be added here. The first test was made by using a horizontal band of sunlight as wide as the aquarium, 1 cm. deep, and a little distance — 5 cm. — from the bottom. Darkening the aquarium momentarily was the means of starting the medusæ swimming up toward the band of light. Usually five or more medusæ were used to begin the experiment. Forty-three per cent. were turned back by the band of light, away from the source; 33 per cent. turned toward the source; and 24 per cent. swam straight through the band of sunlight.[15] Now it was thought that an oblique band of sunlight (similar to Fig. 3) would be more decisive, as one side of the up-swimming medusa would be stimulated, not only more strongly, but in advance of the other. In this case 50 per cent. of the medusæ turned away from the direction of the sunlight and 41 per cent. toward it; 9 per cent. did not respond. A relaxed medusa, allowed to float bell downward, showed a more striking result on touching the oblique band of sunlight. It turned, and after swimming upward a few strokes, floated down, only to do the same on striking the band; the next time it floated through and on emerging below turned in the opposite direction, again away from the band of sunlight, but it continued in a circle which again carried it up through the sunlight.

There is enough difference in methods employed in our experiments to leave no doubt that the medusæ do turn at a sudden transition into strong light, especially when they are in very weak

[15] I am compelled to agree with Morse that the collecting of this medusa in strong sunlight is not a normal reaction, but rather due to previous excessive stimulation of some kind.

light. Morse obtained his result with a limited number of resting or moving medusæ. From the conditions of his experiment we may infer that in some cases he did not get a response in less than three minutes. In my own experiments I rejected any reactions that did not take place within a minute after the stimulus was applied. As to the direction of turning into or away from sunlight there is not so great uniformity. Had I taken a few cases like the one of the relaxed medusa above, I would have gotten high percentages. As it is, I have a large enough result (56 per cent.) to conclude with others that *Gonionemus* turns away from strong light. But on carefully observing this turning it is evident that not in many cases do the medusæ turn in such a way that their continued swimming would take them parallel with the light direction. Not infrequently they turn back into the strong light, as has also been observed by others,[15] or are not turned far enough. Is not Morse's ingenious explanation, given below, also a tacit admission that the mere turning by strong light does not cause a medusa to move parallel to the light direction? This turning, therefore, cannot be considered true orientation, and would not lead to swimming directly away from the source of light. But since it has been shown to be a response to strong light, the question remains as to its use to the organism. In nature the medusæ are probably exposed to sunlight only when they are disturbed and swim to the surface, or when the location of one is exposed as the sun's rays come in a different direction. If now the medusæ were to be oriented and swim away from the light it would take them downward, as Morse[16] has already pointed out. It is to be noticed also that both in nature and in aquaria, the bottom prevents this, and in a measure compels swimming toward the region of lesser illumination if the turning by light has been anywhere within half a sphere. It will be seen that this behavior, repeated, even if no further directed movement takes place, will be helpful in temporary escape from strong sunlight.

Ways of Getting into Optimum Light.

If I have succeeded, so far, in making my position understood the question will naturally arise, How do the medusæ get into

[16] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 455.

favorable light areas? Although Yerkes does not hold it so, I believe he has indicated one way in his observation:[17] "When an individual in swimming about chances to cross from the sunlit region into the shadow, it very quickly ceases swimming and sinks to the bottom." In another connection but bearing out the same point, Morse[18] has this to say — "being in motion almost incessantly, and swimming in all directions, it is obvious that sooner or later they will enter the dark area. Once having entered this area, the stimulating effect of sunlight being cut off, they remain as in a trap." Even if we do not hold that only light stimulates, or that optimum light acts like a trap the explanation may hold.

More recently Morse[19] has described a way in which *Gonionemus* may get from a location that is unsuitable with respect to light to a more suitable one. A medusa was placed in the end of an aquarium through which the sunlight was reflected horizontally. The medusa swam to the surface in characteristic fashion, each time bending its upward course a little farther from the vertical, and therefore away from the source of light. Thus ultimately it got to the farther side of the aquarium, into weaker light. The promising feature of this explanation is that it is based on the peculiar habit of the animal — swimming to the surface when disturbed. The other case that Morse records of a "strong swimmer" moving directly toward the less illuminated end of the aquarium I should consider an exception, as in repeating the experiment I have observed that some medusæ move almost directly to the lighter end after proceeding, by stages, to the darker end. In my trials I have found that about 25 per cent. get to the farther end of the aquarium by the method indicated, but instead of its being a regular method of progression it seemed to me to be characterized by irregularity. This may be due to the fact that Morse seems to have worked with few individuals, whereas I placed a number in the aquarium at the beginning of the experiment and added to these as the experiment progressed, so as to get results representative of more than individual be-

[17] *Amer. Jour. Physiol.*, 1903, Vol. IX., p. 282.
[18] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 454.
[19] *Am. Nat.*, 1907, Vol. XLI., p. 684.

havior.[20] While the majority of the medusæ in the experiment finally reached the less illuminated end of the aquarium they did so by regularly swimming about, resting longer each time they had progressed farther from the light and a shorter time between swimming intervals that again took them toward the light. A few came to rest in the lighter end of the aquarium, as almost always happens in light experiments.

Now if the medusæ do not swim directly toward weaker light and are not turned definitely in such direction, even after trials, at any time, their collecting in weaker light might still be accounted for by the above explanation, if this could be elevated to the dignity of a method. That is, each time a medusa gets into an optimum light it remains longer, and when in an unfavorable light field remains a shorter time, and thus more and more of them will get together in these optimum places.[21]

From the foregoing it will be seen that there are several ways in which *Gonionemus* gets away from the strong light into an intensity best suited to its activities, without the intervention of tropism or "trial." If its only mode of locomotion, or even the chief one, to stimuli were the up-swimming "surface reaction" then it would plainly be "trial," or "motor reaction."

Change of Intensity as a Stimulus.

In Yerkes's earlier statements [22] about the relations of *Gonionemus* to light the words increase and decrease of light intensity are used, but only in his later answer to Morse's criticisms [23] does he make the statement that he has "abundant evidence that change in intensity of light stimulates the medusa." I had experimentally come to the same conclusion.

When the medusæ are at rest darkening the aquarium or shading one with an opaque object, such as the hand, is sufficient

[20] Once I observed a medusa that seemed to follow pretty regularly a movable slit admitting stronger light into a darkened aquarium. Although it was pronounced I could not confirm the reaction in other specimens.

[21] After writing the above I find Mast ("Light Reactions in Lower Organisms, II., Volvox," *Jour. Comp. Neurol. Psychol.*, Vol. XVII., p. 169) has similarly explained the aggregation of *Volvox* in optimum light.

[22] *Amer. Jour. Physiol.*, 1902–3, Vols. VI. and IX.

[23] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 460.

to start movement in a few seconds; again, by throwing stronger light with a small mirror on any medusa it can be made to move about. Indeed, the one or the other of these ways was generally used in my experiments to get motor reactions. Next some experiments were made to determine the height to which *Gonionemus* would swim after a single stimulation by change of light intensity. Eight medusæ were placed in a hydrometer jar 72 cm. deep and 9 cm. in diameter. When they had come to rest in the bottom of the jar they were stimulated by darkening the room until the medusæ could just be seen. They swam to the top, at intervals, somewhat irregularly. The shades were now quickly raised and thus the top of the jar illuminated with strong diffused daylight. Three medusæ swam downward so directly as not to touch the sides of the aquarium — not varying 9 cm. from their course. Five floated down part way and then two turned and swam the last third of the way down. In another trial, out of three at the top one swam down 31 cm. without touching the sides of the glass jar. This, it would seem to me, is in the nature of proof that change of light intensity starts and gravity directs the downward course.

Some Mooted Points.

In regard to the question whether the decrease or the absence of light (darkness) is a stimulus for motion or inhibition there is difference of opinion. Yerkes in his first paper [24] on *Gonionemus* says "Romanes's statement that change from light to darkness is inhibitory of action is not very apt." He adds, it "is merely the absence of any motion producing stimulus." In a later paper on this medusa, however, he [25] says "decrease in light intensity temporarily . . . inhibits activity." Morse [26] from practically the same experiment that Yerkes used concludes that "we have no inhibition of movement in passing from light to darkness. In the dark the stimulating effect of light is absent and hence the movements ultimately cease." Yerkes [27] does not agree with this

[24] *Amer. Jour. Physiol.*, 1902, Vol. VI., p. 445.
[25] *Amer. Jour. Physiol*, 1903, Vol. IX., p. 282.
[26] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 453.
[27] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 460.

criticism but modifies his former statement to say "a considerable decrease causes a more gradual cessation of activity." I have no doubt that he has the correct solution of the question, as shown by the experiments above, when he says — "the change in intensity of light stimulates the medusa." In other words, then, a change in light intensity not only stimulates a resting medusa to move but it may bring a moving individual to rest. This is in accord with well-known facts in the behavior of other animals.

This leads to another point under discussion, *i. e.*, whether or not "the reactions of a swimming organism are different from those of one at rest."[28] Morse[29] believes they are not and supports his contention with an experiment of letting light fall on one half of a medusa resting in the dark; then on the half of a swimming individual. In swimming up both turned from the vertical. It is clear that the discrepancy is based on a misapprehension. To take Morse's own case as an illustration: the light let fall on the resting medusa set it in motion and beyond this, the light produced the same reaction, as it was really in each case falling on a swimming medusa. Therefore Yerkes's statement above is correct.[30]

Surface Reaction.

While there is no doubt that the up-swimming of *Gonionemus* is directed by gravity as stated by Yerkes,[31] nevertheless light seems to be a more important factor than he holds. Indeed I may say it is a necessary concomitant as may be seen from what follows. That it is not directive Yerkes (page 281) has shown by his experiment of using bottom illumination. The medusæ move up to the surface and turn over normally. But casual observation of the upper surface of the water shows that it is sufficiently illuminated from the bottom to allow the medusæ to come to the top. So I substituted lateral illumination through an opening near the bottom of the aquarium. The aquarium

[28] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., Morse, p. 452; Yerkes, p. 462.

[29] *Amer. Nat.*, 1907, Vol. XLI., p. 683.

[30] Other points are covered in foot-notes 3, 10, 15, pages 356, 357, 359, also page 365.

[31] *Amer. Jour. Physiol.*, 1903, Vol. IX., p. 281.

was otherwise darkened on all sides and reflection reduced as much as possible by filtering the water. Now the medusæ swam near the bottom, 8 cm. being the greatest height reached. As a control, bottom illumination was then used and immediately one of the animals swam to the surface and one came near it. Three other experiments were made, and the most striking case was that of one medusa swimming through the light band fourteen times in 72 seconds without reaching top or bottom. When the aquarium was so much darkened that I could not see the medusæ, sudden illumination showed that they had attached by their tentacles to the sides or bottom. Thus the presence of light seems necessary for the regular up-swimming activity of *Gonionemus* and gravity then acts as a stimulus to direct it.

In regard to the part light plays in the surface reaction, Yerkes[32] says: ". . . although light seems to be one of the important conditions for this reaction, it may occur in the absence of light." My experiments just cited show that the last part of this statement is not tenable and that the first part is correct. The chief importance of light in bringing *Gonionemus* to the surface, it seems, is in keeping the medusa negative to gravity.

Morse[33] incorrectly, believing that Yerkes held that light causes the inversion at the surface, denies this, and concludes "the cause for reaction is not evident." Later[34] he explains the inversion the same as Yerkes had previously done[35] by assuming that the apex of the bell is thrust unevenly above the surface. Now as the apex of the bell is heavier we no doubt agree that gravity causes the inversion, though not as a stimulus.

As the inversion at the surface is preceded by inhibition of contraction the question arises whether this is due to strong light. Yerkes[36] and Morse[37] have observed that medusæ do not stop and turn (invert) when made to swim up against a heavier substance than air, such as a board, *a glass plate*, a layer of olive oil, but they continue to swim against these layers until ex-

[32] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 458.
[33] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 451.
[34] *Amer. Nat.*, 1907, Vol. XLI., p. 686.
[35] *Amer. Jour. Physiol.*, 1903, Vol. IX., p. 281.
[36] *Amer. Jour. Physiol.*, 1903, Vol. IX., p. 281.
[37] *Jour. Comp. Neurol. Psychol.*, 1906, Vol. XVI., pp. 450, 451.

hausted. The fact that inhibition and turning do not take place under glass indicates that light does not cause either. The above observations suggested to me that contact of the apex of the bell with air may cause cessation of movement. This idea is apparently supported by holding a layer of air imprisoned under a petrie dish cover some distance below the surface of the water. The animals respond the same as at the surface. Nevertheless, longer observation of the behavior suggests that it is after all, perhaps, nothing more than the recoil of a last ineffective stroke; something as when a person, finding one less step than he expected at the top of a stairway, does not immediately contract his muscles for another step but loses his equilibrium. Light not being the cause of the inversion (with Morse[38]), nor of inhibition, no further discussion is warranted here.

Are the Medusæ Directed by Light Rays?

Morse[39] has decided from an experiment with oblique illumination over the end of a shaded aquarium, because the medusæ collect in the ray-direction-end of the aquarium rather than in the shaded end, that "the direction of the ray of light is the important

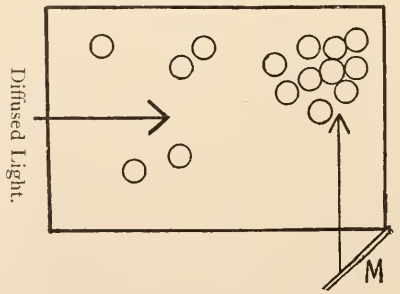

Fig. 2. Top View.

factor in orientation." Has he not left out of consideration another important factor, that of light intensity in the aquarium? Change of intensity has already been shown to be the important stimulus in reactions to light, nevertheless, it seemed worth while to test whether it is ray direction or intensity that determines where the

[38] *Jour. Comp. Neurol. Psychol.*, 1906, Vol. XVI., pp. 450, 451.
[39] *Amer. Nat.*, 1907, Vol. XLI., p. 684.

medusæ will collect. The following experiments were tried: The aquarium used in previous experiments was placed before an open west window at three o'clock in the afternoon, in good diffused daylight. In fifteen minutes the majority of the medusæ had retreated to the end of the aquarium away from the window (Fig. 1, p. 356). Now a large mirror (*M*, Fig. 2) was placed vertically against one corner of the aquarium away from the window, so as to throw light across the end of the aquarium. In fifteen minutes most of the medusæ had collected in the opposite corner of the aquarium away from the window and where the light was least intense. The mirror was now placed at this corner and record again made in fifteen minutes. Many of the medusæ were scattered about, but again there was a larger number collected in the

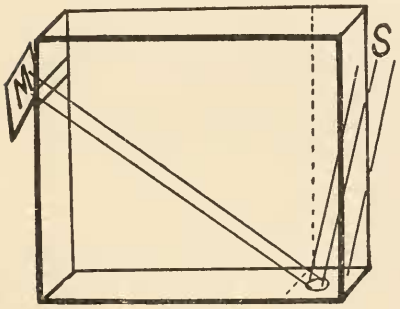

FIG. 3.

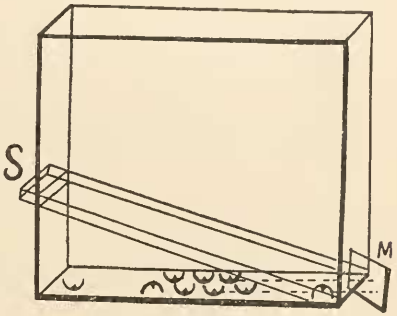

FIG. 4.

corner farthest from the sources of light. In two other experiments a sort of spot-light effect was produced, by having the aquarium darkened except at one end, and a vertical strip (Fig. 3) at one side next the open end so as to throw sunlight across the open end diagonally. There was also a slit near the top of the closed end of the aquarium for a band of sunlight or a mirror beam. The medusæ gathered farthest from the source of light when sunlight was passed through the end of the aquarium. Now crossing this sunlight by a beam from a small mirror, through the slit, made the medusæ leave the place where the light overlapped — where it was more intense. The position of the sunbeam and mirror was reversed with a corresponding result. In this case the mirror was held high enough so that its circumscribed area of reflected light fell on the farther corner of

the aquarium, leaving a place of high intensity where the band of sunlight fell, but not so high as that where the two overlapped (Fig. 3). Other portions of the aquarium seemed to be of too low intensity, as the medusæ remained in the lighted portions. The effect of relative intensities was then seen by lowering the mirror so that its beam was projected horizontally across the band of sunlight. Again there were more medusæ in the corner farthest from the two sources of light (compare Fig. 2). In the final test, sunlight was admitted through a slit near the bottom of the aquarium and the mirror placed back of the aquarium in such a position as to throw reflected light obliquely against the sunlight, as it were (Fig. 4). Now the medusæ collected some distance from the back of the aquarium in the region of lesser intensity. Finally, that it is relative intensity and not ray direction is also shown by a former experiment (page 359) where medusæ turned away from an oblique band of sunlight in a darkened aquarium. They turn nearly as often toward as from the source of light. Ray-directing, seems to me, out of the question.

Summary.

1. The medusæ do not usually direct their movements to favorable locations but continue swimming at random until they come into an optimum environment, where they settle down.

2. Intense light turns medusæ away, thus avoiding injury.

3. Change of light intensity is the stimulus for reactions to light. In pronounced decrease, the change of intensity causes inhibition.

4. Relative intensity in the field, not ray direction, determines the place of rest.

5. Light is necessary for the up-swimming activity, though not directive — this being due to gravity.

6. Contact of the bell with air and the accompanying recoil probably causes the inhibition that precedes inversion of the bell at the surface.

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BIOLOGICAL BULLETIN

NEW AND LITTLE KNOWN HYDROIDS OF WOODS HOLE.[1]

CHAS W. HARGITT.

During the summer of 1907, while engaged upon certain problems associated with the work of the biological survey carried on at the Fisheries Laboratory, I described several hydroids, some new, others more or less rare, in a paper published in the BIOLOGICAL BULLETIN, January, 1908. During the following summer I was fortunate in finding a few others which, like the former, were in part new and in part hitherto unknown within the locality, and in one case at least, wholly new to American fauna. In the following account will be found such descriptions as seem called for in order to bring them definitely to knowledge as integral factors of the hydrozoan fauna of the region concerned.

CLADOCORYNE FLOCCOSA var. SARGASSENSIS.

In a mass of *Sargassum* which was picked up during the summer of 1907 in Vineyard Sound, bearing a rich hydroid fauna, I found a very minute hydroid which at first greatly puzzled me. It was intricately associated with other species, particularly with *Aglaophenia minuta*, and at first seemed to be a sort of nematophoric accessory of this hydroid, the small round heads of young specimens bristling with nematocysts having but little resemblance to an independent hydroid. But a more extended examination brought to light other and larger specimens, and soon it was found that the thing under examination was beyond doubt a very minute and apparently unknown species of hydroid. A series of developmental stages were found giving all conditions, from minute buds just arising from the stolon-

[1] Contributions from the Zoölogical Laboratory, Syracuse University.

iferous base to others with mere buds of tentacles, with still others having growing tentacles from the base of a definite hydranth on to the fully developed hydroid with full complement of tentacles, etc. Fig. 1 shows the hydroid enlarged ten diameters, while Fig. 2 shows the hydranth greatly enlarged to show the peculiar branching and knobbed tentacles. With this much clear it was not difficult to trace its generic affinities under *Cladocoryne*, Rotch.[1] But it was doubtful as to its specific relations. Rotch had described a species, *C. floccosa*, found at Herm, near Guernsey, having a habitat on stones, and being 6–12 mm. in height. Perrier has also described a species, *C. simplex*, found on *Sargassum*,[2] but I have not had access to Perrier's book, and so am unable to form any definite notion of that species.

FIG. 1.

The present species is very minute, being only 2–4 mm. in height and differing more or less as to other features. I have suggested for it a varietal distinction, proposing the name *sargassensis*, as indicative of its habitat. The following characters are diagnostic:

Trophosome. — Stems mostly simple, occasionally branching slightly, rising from a reticulate hydrorhiza. Hydranths relatively large, spindle-shaped, with elongated hypostome similar to that of *Pennaria*. Tentacles about twelve, variously branched and definitely knobbed, and disposed in some three verticels over the body of the hydranth. These tentacles are peculiar and thoroughly distinctive, both in structure and development. A second series of oral tenacles, about six or seven in number, are simple, with knobbed ends, and surround the mouth. All are richly packed with nematocysts.

The perisarc, both of stem and hydrorhiza, is rather dense and irregularly annulated.

Gonosome. — This is wholly unknown, in the present specimens at any rate.

Habitat and Distribution. — The present is the only time I have seen the species. As stated before it has its habitat on

[1] *Ann. Mag. Nat. Hist.*, March, 1871, Vol. VII., p. 227; Allman, "Gym. Hydroids," p. 38.

[2] Cf. Billard, "Exp. Talisman," p. 161.

floating *Sargassum*. I have hunted carefully over later collections of gulf weed but without finding trace of it.

CALYPTOSPADIX CERULEA Clarke.

On August 7, 1908, I found growing on the sides of the steamer Fish Hawk, at Woods Hole, several fine colonies of this hydroid, originally described by Clarke,[1] and so far as I am aware has

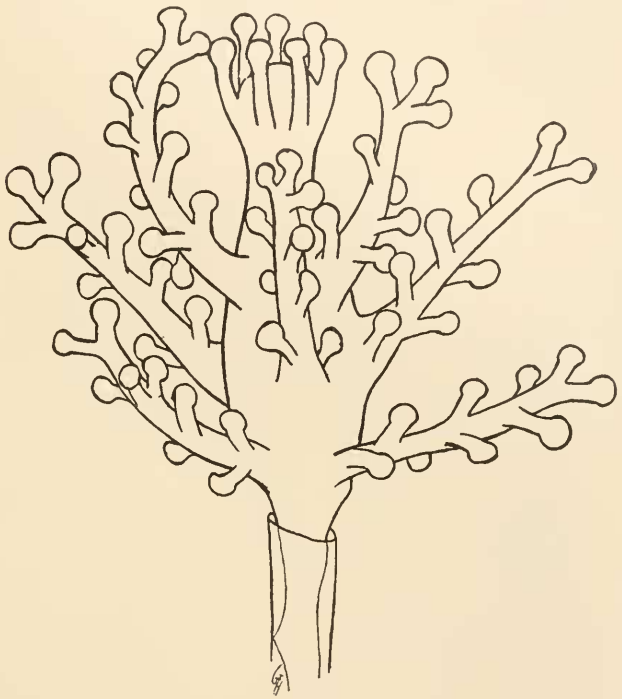


FIG. 2.

not since been a subject of record. In general aspects and size it resembles *Bougainvillia*, and was in the present instance thought to be that hydroid. A closer scrutiny soon revealed its marked differences.

Its original description from Chesapeake Bay, and its occurrence on the Fish Hawk, which had only a month previous come from Norfolk, at once suggested the probability of its having been thus transported to this locality. It is not strange, there-

[1] *Mem. Bost. Soc. Nat. Hist.*, Vol. III., 1882, p. 136.

fore, that I made the following entry in my notes at the date above mentioned: "This is a fine illustration of the importance of ships as a means in the distribution of organisms." On the following day it occurred to me to look about the docks at which the steamer was moored as to whether any signs of the hydroid might be found on the piles; and somewhat to my surprise colonies were found at several points, some of them quite remote from the ship. Immediately the query arose, Did the Fish Hawk bring the hydroid, or had it found a place on the ship from contiguous piles of the dock? The smaller and younger conditions of colonies on the ship suggested the latter alternative, but still with the prepossession of theory strongly inclining to the former. An examination of the outer side of the ship showed an almost entire absence of the hydroid, which still further emphasized the doubt as to the ship's relation to the matter of distribution. The matter found a final solution so far as the *present* issue was concerned when on August 10th Mr. Vinal Edwards having at my request brought a few hydroids from Wareham bridge at the upper arm of Buzzards Bay, and I found among the material fine colonies of the same hydroid. This of course ruled out the Fish Hawk so far as the present case was concerned, for the last habitat was entirely beyond the reach of the ship as a means of transportation.

During the current season, 1909, I looked several times at the fisheries docks for colonies during July and early August, but in vain; but again I was able to obtain luxurious colonies from the Wareham locality. This clearly established the fact that the species is thoroughly established as a permanent feature of the local fauna. But the matter as to *how* and *when* it became established must be a subject of much uncertainty for the present. That it has been established for any considerable time I seriously doubt, having been collecting throughout the region more or less assiduously for many years without previously finding any trace of its presence.

The hydroid is a large and beautiful species, the bluish color of the female gonophores making it strikingly different from almost all other species of its character. Fig. 3, copied from Clarke's paper, gives a fair idea of the main features of the hydroid.

CLYTIA VOLUBILIS. Fig. 4.

On floating masses of *Sargassum* were found prolific colonies of a hydroid which had many of the characteristics of *Clytia johnstoni*, and which I took for a time to be that species, though recognizing certain features which differed from it. During the current summer I took at Harpswell, Maine, what proved to be very typical specimens of the species, and which upon comparison with the former showed very marked and constant differences. I was therefore forced to reconsider its specific relations. In

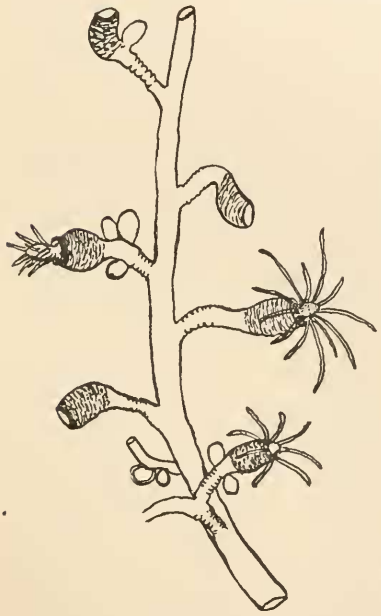


FIG. 3.

FIG. 4.

doing this I had occasion to compare it with specimens taken at Naples several years ago, and which I had then considered as *C. johnstoni*. The two species had much in common, indeed differed hardly more than might species from remote localities. A review of the literature brought to light the fact that certain authorities have considered the two above named as identical. For example, in his monograph "Die Hydroiden des k. k. naturhistorischen Hofmuseums," Marktanner-Turneretcher has thus treated them, giving preference to the earlier name of Ellis and Solander.

A comparison of the characteristic specimens of *C. johnstoni* taken at Harpswell with the Woods Hole and Naples specimens has led me to consider both as entitled to specific distinctness, and I am therefore designating the local species as *C. volubilis*, and believe the Naples specimens to be the same. The following features are diagnostic :

Stems usually simple and unbranched, 2–4 mm. high, annulated at proximal and distal ends, occasionally indefinitely annulated throughout. Hydranths relatively large, with 20–24 stout tentacles, and with a prominent hypostome, more or less trumpet-shaped in expansion. Hydrothecæ broadly campanulate, not very deep, and with about 10–12 shallow rounded teeth, in some specimens the margins hardly more than undulate.

Gonangia borne on the reticulated hydrorhiza, rather large, and with very short plain pedicels. An interesting feature was the fact of a remarkable variation as to the aspects of these organs. Most were rather smooth, oval structures ; but in not a few cases they were strongly corrugated throughout, and examples showing all phases of intergrading in this particular were easily found.

It may be well in this connection to call attention to a species of *Clytia* described by Congdon from Bermuda,[1] *C. simplex*, which has features in some measure intergrading with the one under review. I have not seen Congdon's type specimens, hence have only his general description as a guide. It will be seen that his specimens average considerably larger than my own, and the hydrotheca is given as longer, and with deeper teeth, still it might be worth an attempt to critically compare the types of these several species with a view to ascertaining just what grounds might be found bearing upon their interrelations.

Clytia cylindrica Ag.

On at least two occasions recently I have taken this beautiful little hydroid. While at times it may be found in considerable numbers, it does not seem to be especially common, though this may be due in part to its very small size. In height the simple stems are from 1–1.5 mm. ; the hydrothecae about 0.5 mm.

[1] *Proc. Am. Acad. Arts and Sci.*, Vol. XLII., p. 471, 1907.

long by about 0.2 mm. broad; they are cylindrical in form, with about 8–10 sharp, deeply cut teeth. Gonangia are elongate, more or less cylindical, smooth, borne on delicate pedicels ringed at proximal and distal ends. The hydranths are extremely delicate, and with delicate orange to reddish tints just below the tentacles.

Opercularella pumilla Clark.

Among a few hydroids collected in March, 1908, by Dr. F. B. Sumner were found a very few specimens of this species, a record of which is important since I can find no evidence of its occurrence since that of its original description by Clark.[1] He records having taken it at Portland, Maine, and off Montauk Pt., Long Island. The related species, *O. lacerata* Hincks, he records from New Haven, Conn. Clark expressed some doubt as to whether his species really came under the genus to which it was assigned, and Nutting has expressed doubt as to the validity of the species, believing it probably identical with *O. lacerata*. My own specimens conform very closely with Clark's description and figures. It is a most beautiful and delicate little hydroid. Stems and branches are annulated throughout. No gonangia were present on my specimens.

Obelia congdoni, n. sp.

On several occasions recently I have taken from floating gulf weed at Woods Hole an *Obelia* which, while apparently closely related to *O. hyalina* Clarke, differs in several important features, as will be pointed out later.

Congdon has recently described a species from Bermuda, which he referred to Clarke's *O. hyalina*,[2] but which I am convinced is identical with the species under consideration, and which seems to me to be an undescribed species.

Congdon's description and figures are sufficiently accurate to obviate necessity for any considerable details in this connection (cf. *op. cit.*). A few points which seem to be in rather sharp contrast with Clarke's species may be given.

According to Clarke[3] the "branches of the stem arise in the

[1] *Trans. Conn. Acad. Sci*, Vol. III., pp. 61–2.

[2] *Proc. Am. Acad. Arts and Sci.*, Jan., 1907.

[3] *Bull. Mus. Comp. Zoöl.*, Vol. V., 1879, p. 241.

axils of the hydrothecæ." This I do not find to be the case in the present species. Again, according to Clarke, the "gonangia are small, about twice the length of the hydrothecæ, rounded off at the distal end, with a simple spherical, terminal opening which stretches across the distal end." On the contrary, the gonangia are large, about four times the length of the hydrothecæ, and the opening is not simple, but there is a terminal neck with everted rim. It should also be said that in contrast from Clarke's species in which the colony is said to be "about 12 mm. in height, and but little branched," in the present case the colony is from 20 to 30 mm. in height, and much branched.

Gonosome. — The medusæ when liberated have 24 tentacles, but others are rapidly acquired and within ten or twelve hours many specimens have from 30 to 36. The general aspects of the medusa are distinctively obelian ; there is the eversible bell, the squarish manubrium at base, with rounded oral portion, with two otocysts in each quadrant.

Regarding the species as new, and in deference to Congdon's description, I suggest as its specific designation *Obelia congdoni.*

Calycella syringa.

This species is neither new nor rare in this region. Reference is made to it for the purpose of calling attention to certain features of habitat and variation which seem of some interest and importance. Nutting refers to it as "found abundantly in the Woods Hole region, growing over all sorts of plant-like marine organisms, especially other hydroids." This statement I am able to confirm, though with a single qualification, namely, its seasonal oscillations. I have found it rather *uncommon* during the midsummer season, and have never found it actively propagating at this time by sexual modes. In early spring — March to May — it seems much more abundant and immense colonies with prolific crops of gonangia are not rare.

Another feature calls for some attention, namely, the variable size and aspects of the species in midsummer. At this time specimens found by me have been invariably of dwarfed character, so much so that for some time I was rather inclined to consider it as a distinct species. Typical specimens taken in

spring have the distinctive elongated and spirally annulated pedicels and large hydrothecæ. But specimens taken in summer, so far as my observations have gone, are uniformly and constantly small — only about one fourth that of typical specimens, and have extremely short pedicels, with only one or two annulæ, or with none. I was not unaware that Clarke [1] had referred to certain variations in size, but he made no special reference to it save as an exception. It was only after careful search among colonies of typical specimens that I was able finally to find an *occasional* specimen of this dwarfed character. I have satisfied myself that it is but another instance of that tendency to seasonal variation which is well known in other cases. It is well, however, that it

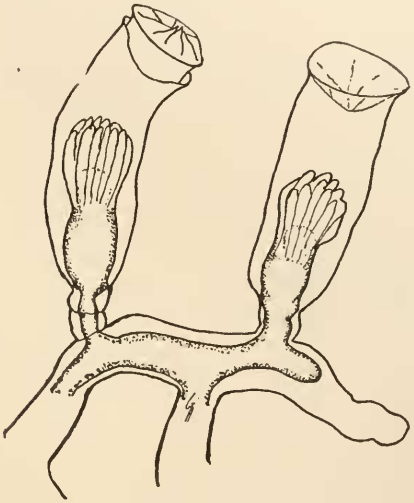


FIG. 5. × 100.

be emphasized, as well as the further fact that at certain times *dwarf features* are *distinctive* and *constant.* Fig. 5 shows some of these dwarfs enlarged.

One other feature may be referred to in connection with this phase. Clarke called particular attention to the appearance in certain hydrothecæ of this species of a "wide ring, oramented with from ten to fourteen longitudinal markings, which rises for some distance above the rim and on the summit of which there is borne either an operculum or another ring; in some cases

[1] *Trans. Conn. Acad. Sci.*, Vol. III., p. 66.

there are as many as four of these rings with an operculum at the summit." Such series of rings I have found to be rather common; but it has not been possible to distinguish, even with high powers, the "ornamental markings" to which Clarke makes reference. The surface of these secondary, or additional rings is quite as devoid of such markings as is that of the original hydrotheca itself.

CALYCELLA NUTTINGI, n. sp. (Figs. 6, 7.)

Growing upon colonies of the bryozoön, *Bugula turrita*, taken at the fishing grounds off Sankety, and later at Woods Hole, and even still later at Harpswell, Maine, I have found a microscopic species of *Calycella*, which seems to be undescribed. It is hardly more than one tenth the size of an average specimen of *C. syringa*, and differs in other respects as well. Its very minute size may probably account for the fact of its having been overlooked in

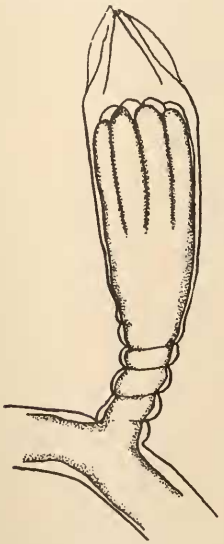


FIG. 6.

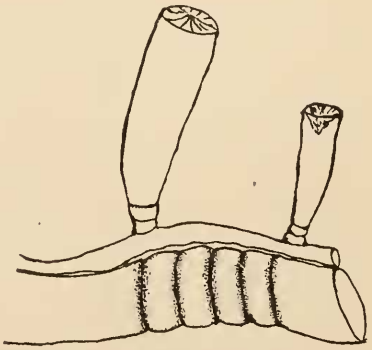


FIG. 7.

spite of continuous collecting throughout the region for many years. The following characters are diagnostic of the species:

Trophosome. — Colony composed of a creeping, filiform stolon, slightly, if at all, reticulated, from which at very irregular intervals arise the hydrothecæ. These are tubular, though not quite

cylindrical, gradually widening from base to margin, as shown in Fig. 7, and are without appreciable constriction at base where it articulates with the short, annulated pedicels, the annulations occasionally extending some distance (rarely over entire body), on the thecal walls, giving the impression of complete annulation when viewed obliquely. The hydrothecæ are very delicate, often collapsing at the distal ends when being prepared for mounting. There is a definite operculum, which often appears plaited, the individual valves being more or less difficult to distinguish. I have not determined their exact number with any degree of certainty. In many cases these valves exhibit the same aspect of inversion as is the case with *C. syringa*, but I have not found the presence of secondary rings or other marginal duplication as in the latter species. Total length of pedicel and theca 0.2–0.3 mm. or an average of about 0.25 mm., by about 0.07 mm. in diameter.

Hydranth extremely small and delicate; body elongate, cylindrical, with conical hypostome; tentacles very delicate and thread-like, usually ten in number, occasionally eight.

Gonosome unknown. The examination of many colonies from various localities failed to discover signs of gonangia. It may be probable that like *C. syringa* this species has its breeding season at some other time of year.

Habitat. — Found only associated with other hydroids, or similar organisms, *e. g.*, bryozoa, and hence is probably of commensal habit. No evidence was found indicating parasitism.

It is a pleasure to name the species, with his consent, in honor of my friend and distinguished student of hydroids, Professor C. C. Nutting.

KERATOSUM COMPLEXUM, n. gen. and sp. (Figs. 8–10.)

On three successive summers there has been taken an organism at Crab-ledge which was variously assigned to the Porifera, Bryozoa, and finally came to the writer. A glance at Fig. 8 will show how little there is from a superficial view to suggest hydroidean affinities. Indeed it was only after sections had been made, or maceration and dissection of the thing, that its true relations became evident. And it was only after considerable re-

search that its generic relations were even approximated. In 1892 Levinsen described a hydroid from Greenland (Meduser, Ctenophorer og Hydroider fra Grönlands Vestkyst),[1] which seemed to have much in common with the one here under review. He had described it as a new species under the genus *Lafœina*, Sars, naming it *L. maxima*. At first it was thought the present

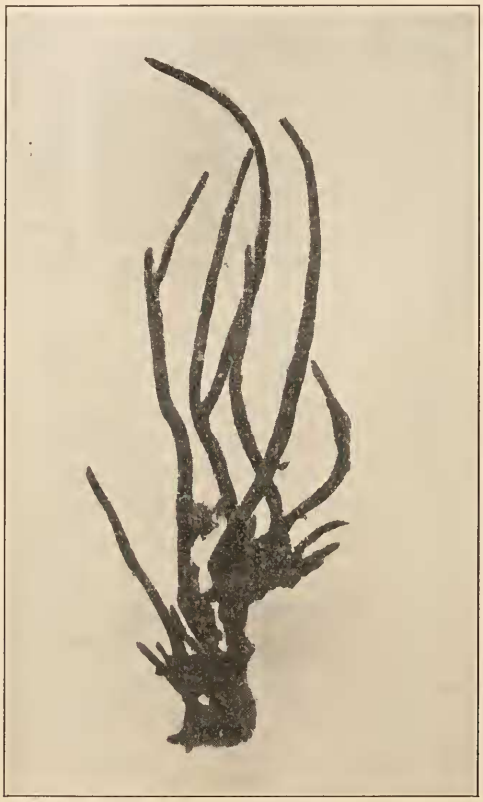


FIG. 8. Photograph of colony ⅔ natural size.

species was probably identical with it, but when one undertook to work out details of morphology it became more or less certain that it not only was not the same species, but that, moreover, it could hardly belong to the same genus, if, indeed, there might not be the necessity of establishing for it a new family.

[1] "Saertryk af Vidensk. Meddel. fra den naturh. Foren.," 1892.

The genus *Lafœina* was established by Sars (Bidrag til Kundskaben om Norges Hydroider)[1] for a very minute hydroid found on stems of *Perigonimus*, the chief generic character of which was the presence of minute urticating organs, or nematophores, unlike any before known. Levinsen's description is rather inadequate, and his figures not altogether satisfactory, but to the writer there seems to be so comparatively little in common between his species and that of Sars, that it may be doubtful whether it should

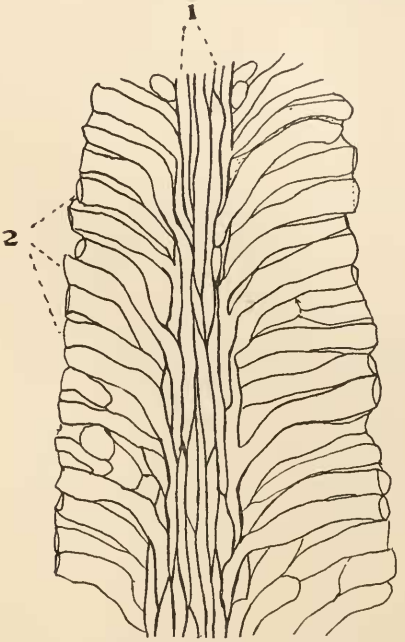


FIG. 9. 1, Axial tubes; 2, hydrothecæ.

not have been placed under a new genus. Be that as it may, it seems very sure that the present one must find different generic housing. For example, in Sars' genus the hydroid has a reticulate hydrorhiza, and Levinsen describes something of the sort for *L. maxima*, but in the present species while there may be comprised something of the sort, it would be more correct to describe the complex stem as arising from a dense, sponge-like base, etc.

Concerning the family relations I am not disposed in this connection to enter into any critical review. While the Perisi-

[1] "Saerskilt aftrykt Selsk. Forhandlinger" for 1873.

phonidæ would be the only one under which it might be placed, still the family as at present defined, according to Allman (Hydroida, Part II., p. 32),[1] would by no means provide for the species. For example, while there is an axial tubular mass, as shown in Fig. 9, there is no single one of these which bears the hydrothecæ as called for by the definition referred to. However, for the time being the species may be left under this family till such time as adequate revision may be undertaken, when the needed modifications may be provided.

As already intimated, it seems necessary to institute a new genus as well as species for our hydroid. For the genus characters the following are designated as diagnostic :

Colony sponge-like, both in general aspect and in the texture of stems and branches, as well as in growth-habit. Looked at from a short distance it resembles very much our common sponge, *Chalina arbuscula*, in almost every particular. Hence the proposed generic name — *Keratosum*. The stems arise from a disk-like spongy base and branch much after the manner of "finger sponges." These are composed of a complex and intricate mass of siphon-like tubes which ramify and anastomose irregularly, and from which arise hydrothecæ, and nematophoric organs, the latter with thecoid terminal structures similar to the former, the whole cemented together by a dense sponge-like felt of very tenacious and resistant character. Longitudinal and transverse sections of stems or branches show them to be composed of the following parts : (1) a central, axial portion, made up of more or less parallel, anastomosing tubes ; (2) a peripheral portion, composed chiefly of hydrothecæ and what may be termed nematothecæ ; (3) ramifying strands of cœnosarc, which seem to interpenetrate the elements of the peripheral zone. Figs. 8, 9 and 10 will show both the surface aspects as well as sectional views just mentioned.

Concerning specific diagnosis it must be regretted that the physiological state of the hydroid was such as to afford but meager characters of specific nature. The organism in all the specimens collected seemed to be in a state of hibernation, or better, perhaps, *æstivation*, no hydranths or similar organs being

[1] "Report Chall. Exped.," Vol. XXIII. (part 70), p. 32, 1888

distinguishable. Hence such organs as tentacles, gonophores, etc., which afford important specific characters, were wholly lacking. I had at first attributed this condition to bad preservation; but collections made at two subsequent seasons, in each case care being taken to preserve by approved methods, have convinced me to the contrary. It seems highly probable that this hydroid during the summer season is in a state of suspended animation, so to speak; a condition quite common among hydroids at various seasons. It must suffice in this connection to make brief reference to a few features, as hydrothecæ, etc. As shown in the figure, the hydrothecæ are tubular structures, arising from the axial tubes by rather narrow necks, and extending

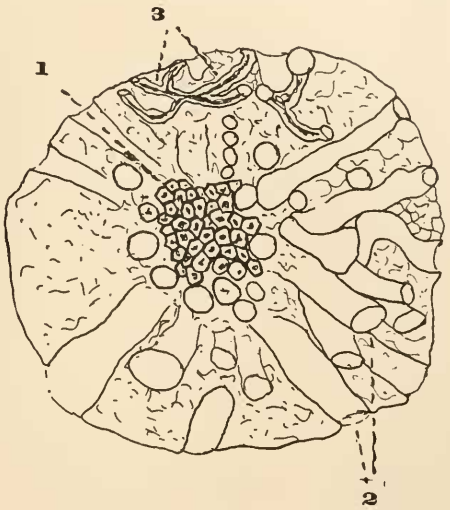


FIG. 10. Cross-section of stem. 1, Axial tubes; 2, hydrothecæ; 3, cœnosarcal strands.

upward and outward, becoming more or less curved, and opening to the surface by somewhat oblique mouths. While in many cases there seemed to be opercular-like folds at the thecal openings yet they were difficult to definitely demonstrate or describe. As to size hydrothecæ averaged about 0.7 to 1 mm. in length, by about 0.12 mm. in diameter at median portion, somewhat larger at mouth. In no case were gonangia distinguishable, nor evidences of germ cells. This might be expected as to the last, but if gonangia are an organic part of the skeleton one might

expect some trace of them in some specimens, at any rate. But none could be recognized.

Nematophores were distinguishable, and in a general way seemed similar to those described by Levinsen. They are elongated structures, cylindrical in shape, and with terminal thecoid organs which are smaller than the hydrothecae, much smaller indeed, but with no peculiar or distinguishing features. In many cases the definite organization of the nematophore was distinguishable, and the knobbed heads were found loaded with numerous nematocysts which measured about 0.015 to 0.02 mm. in length by about one third of this in diameter. In shape the nematophores may be designated as elongate-clavate ; and are probably protrusible in life beyond the nematothecæ as organs of defense, or offense, according to circumstances.

In connection with the account of the morphology there should have been mentioned a matter of interest, as well as of difficulty, namely, that concerned with the attempts to dissect and separate the elements of the complex stem structure. The usual resort to boiling with potash or caustic soda, while affording some aid in clearing out the organic contents of the tubes, afforded very small aid in isolating the elements. Even when macerated for hours or days in strong solutions, or after prolonged boiling, so far as my own efforts were concerned, the macerating processes availed but little. And when resort was had to javelle water the consequences were worse, for with that agent both the cement substance and the chitinous perisarc itself were attacked about equally, and the end was, naturally, the disintegration of the entire mass.

I was interested to find a similar experience recorded by Allman (*op. cit.*, p. 47). Of the adhesion of the tubes of *Grammaria* he says : "So intimate is this adhesion that I have found no treatment, even prolonged boiling in caustic potash, of in any way overcoming it. *Grammaria* in this respect presents a striking contrast to *Cryptolaria*, as well as other genera of the Perisiphonidæ, in all of which maceration in a solution of caustic potash so weakens the adhesion of the tubes to one another that they may then be easily separated by the dissecting needle."

All in all, we have in this hydroid one of the most interesting,

and in some ways anomalous, of this remarkable group of organisms. No name compatible with the rules of nomenclature would in any measure serve to more than hint somewhat of this; hence in proposing for it the above title — *Keratosum complexum* — it may be presumed to be modestly christened!

It is a pleasure to acknowledge obligations to Dr. R. C. Osburn for aid in securing material of this species.

Syracuse University,
September 15, 1909.

AN ECOLOGICAL STUDY OF THE PLANKTON OF SHAWNEE CAVE.

With Notes on the Cave Environment.[1]

WILL SCOTT.

With Three Figures.

During the year beginning September 7, 1907, the speleological fellowship of the department of zoölogy of Indiana University was held by the writer with residence on the Cave Farm of the University, three miles east of Mitchell, Ind.

A preliminary examination of the cave stream revealed the presence of considerable plankton. A systematic collection of the plankton was immediately begun and after some time a quantitative method applicable to the cave was developed.

The organisms constituting the plankton were found to be epigean forms. It developed that the seasonal distribution of the cave plankton is different from that of epigean streams and lakes. Its maxima and minima seemed more closely related to stream level than to any other factor of the environment.

These facts led to an examination of the surface of the region overlying the cave, in order to determine if possible the source of the plankton, and the relation of its source to its distribution in the cave. It was found that the plankton is derived from ponds in sink-holes of a particular type, and that its source is a primary factor in determining its distribution.

After the plankton enters the cave it is modified in various ways by its new environment. The cave environment is divided into two distinct regions, the terrestrial and the aquatic. These two regions are alike in that there is an absence of light in both, and that each influences the temperature of the other. They are also both affected by the form of the cave.

Some of their differences aside from their primary ones are: (1) the temperature of the air approximates the temperature of the walls of the cave very soon after entering it, while the tem-

[1] Contribution from the Zoölogical Laboratory of Indiana University, No. 104.

perature of the water approaches this constant slowly, (2) the rate of the air current in this cave is determined by the outside temperature, while the rate of water current is determined by its level, which in turn is determined by rainfall. Since the terrestrial region affects the plankton-inhabited aquatic, both regions need to be considered.

A careful examination of the bed of the stream was made for sessile and bottom-inhabiting forms with negative results.[1]

Previous Work. — Previous studies on the microörganisms of caves have been confined for the most part to the enumeration and description of species. Claus, Schmeil and Joseph have investigated the zoöplankton of some of the European caves and have recorded the species.

In this country records of microörganisms inhabiting cave water have been made by Tellkampf ('45), Packard ('89), Banta ('07) and others. Kofoid ('99) has described a towing net collection taken in Echo River, Mammoth Cave, by Eigenmann. This catch contained twenty species. Ulrich ('02) reported twenty-one species from the water of an artesian well at San Marcos, Texas. Among these were two species of cyclops, both of which he regarded as new.

The Cave. — The cave[2] in which these studies were made is at present a rather simple tube with a few side passages. A stream of some size flows through its entire length. The openings of the cave are in Section Four (4), Township Three (3) North, Range One (1) East. These openings are five in number. The

[1] I am greatly indebted to Dr. C. H. Eigenmann, professor of zoölogy, for his helpful criticisms and the loan of literature from his private library; also to Dr. Charles Zeleny, associate professor of zoölogy, for valuable suggestions. Messrs. F. C. Greene and N. E. McIndoo assisted in surveying the cave. They are entirely responsible for section II.

[2] The upper end of this cave is not known. It has been explored to a point about two miles above the outlet. At this point a mass of fallen rock nearly closes the passage. Mr. N. E. McIndoo has crawled through this passage, but it is too small to admit the boat. A stream, which drains a valley some two miles long, flows into an underground passage about four and one half miles southeast of the outlet of Shawnee Cave. This is known locally as "Mosquito Sinks" and is indicated by Newsom ('01) in section sixteen, township three, range one north. Possibly this is the upper limit of the cave. The openings where the stream "sinks" are too small to be explored. Five hundred wooden blocks, which had been soaked in paraffine, were placed in these openings, but none has been taken within the cave, to date.

lower one is the outlet and is known as Shawnee Cave. The four other openings have been formed by the collapse of two sections of the roof and are known as, Lower Twin Cave, Upper Twin Cave, Lower Dalton Cave, and Upper Dalton Cave, respectively (see map).

Vertically the cave is located in the Mitchell limestone, and has been formed by solution along seams in the rock. These seams follow approximately cardinal directions and hence cross at right angles. The result of this is that much of the cave consists of straight passages at right angles to each other. The erosive action of the stream being much greater in the eddies at the turns than in the straight passages deep pools are formed at these points.

Obstructions. — The cave stream has been obstructed more or less completely at four points in the region that I have explored. The upper obstruction has been formed by the collapse of the roof, possibly below a sink-hole, between the Dalton caves (I–36, 37).[1] The second in a similar manner between the Twin caves (I–32, 33). A large part of the upper dam has been removed by solution and erosion, so that the stream flows over it.

At the Twin caves the obstruction is complete at ordinary stages of the stream. It occurs at a right-angled turn in the cave. A new passage is being formed cutting off the obstructed corner. The lower end of this new passage is a few feet below Lower Twin Cave, the location of its upper end is not yet positively determined. The passage is too small to be explored and insufficient to accommodate the stream in times of flood; in such times, the water flows over the obstruction between the two caves and resumes its original course.

The third obstruction is 1,400 feet below Lower Twin Cave at the so-called "Big Room" (I–15, 20). Here a number of old caves crossed the present stream at a higher level. The strata between these two cave levels fell and for a time completely dammed the stream. Deposits of gravel and clay were then formed above this point. In the side passages that are protected from erosion by the cave stream, the deposits still reach the roof. Much of the obstruction and resulting deposits have been removed by the cave stream.

[1] Numbers refer to map.

Eight hundred feet below the "Big Room" is the fourth obstruction (I–13). The roof has fallen from some cause that I was unable to determine, and has completely dammed the stream. A new channel has been formed from this point to the outlet, except for a distance of 40 feet, where it flows through an old cave. The direct result of these obstructions is the formation of pools having a maximum depth of about 10 feet.

Elevation. — The outlet of Shawnee Cave is 40 feet above the level of White River which is about two and one half miles distant. The Lower Twin Cave is 30 feet above Shawnee Cave and Upper Dalton Cave is 10 feet above Lower Twin Cave. The gradient of the stream in the lower part of the cave is 40 feet to the mile. It falls very rapidly from the outlet for about 800 feet, and then has a gradient of about 12 feet to the mile. Probably this slight gradient and the close approach of the stream to local base level prevents the stream from finding a lower level when obstructed, thus causing the effect of the obstruction to continue.

As a result of the pools at the turns and above the obstructions, the water in the cave may be divided into a *constant* which is the amount of water in the pools at all times and a *variable* which is the amount of water flowing through the cave. The ratio between the constant and the variable is much greater in the lower (ordinary) stages of water than in times of flood. At ordinary stages of water, it requires a given particle of water much longer to pass through the cave than if the cave were a straight tube.

Five hundred wooden cubes which had been soaked in paraffine were put in at Lower Twin Cave, and a trap of "hardware" netting (one half inch mesh) was set at Shawnee Cave. Fourteen days later the first blocks were caught at Shawnee Cave. In times of flood the current in very rapid. Just what the rate is could not be determined as no trap could be designed that would withstand the terrific force of the stream at Shawnee Cave. Certainly not more than a few minutes are required for water to pass from Twin Cave to Shawnee Cave in times of flood.

It is evident, then, that the plankton of the cave is subjected

to the cave environment for a much longer time during the lower stages of water than during the higher stages. This has a marked effect upon the number of species and the number of individuals constituting the plankton at the different stream levels.

Light. — This cave is like most others for the greater part of its length, in that there is an absence of light. It differs from most others, in that the stream is illuminated at the points where the roof has collapsed. The stream is fairly rapid in the sections exposed to light, so that the illumination is of short duration. The illumination is strong between Dalton caves and scarcely more than twilight at low stages of the stream between Twin caves. These short exposures to light may enable some of the zoöplanktonts to feed and cause some carbon assimilation in the phytoplanktonts. The effect is probably very slight.

Temperature. (*a*) *Air.* — The temperature of the air of the interior of the cave is between 52–56° F. throughout the year. The temperature has been recorded for the last two years on a thermograph stationed fourteen hundred feet below Lower Twin Cave. The variation exceeds the error of the instrument slightly. Where the air flows out at an opening it differs in observed cases less than one (1) degree Centigrade from the temperature of the interior. Inflowing air assumes the temperature of the air in the center of the cave gradually.

On September 7, 1908, a Centigrade thermometer carried into Lower Twin Cave showed that the interior temperature was reached 428 feet from the opening. During extreme temperatures above ground this point would be farther from the opening.

(*b*) *Water.* — The water temperature varies much more than that of the air for obvious reasons. During low water the temperature of the water approaches the temperature of the walls of the cave. During a flood, it varies toward the temperature of the water outside the cave. Floods, then, cause the temperature of the cave water to lower in winter, to rise in summer, but affect it slightly when the outer temperature is near 54° F. The variation is much less in summer than in winter, because the summer floods are not so great.

In every flood observed, the extreme water temperature occurred about twenty-four hours or more after the crest of the

flood. This may be due to the influence of the residual water in the cave (see Table I.).

TABLE I.

SHOWING THE RELATION OF FLOOD TO TEMPERATURE OF CAVE WATER.

Winter.

Date.	Water Temp.	Outer Temp.	Crest of Flood.	Rainfall.
Dec. 12	11.6° +C.	< 0° C.	Crest	1.48
" 13	11.5° +C.			
" 14	8.7° +C.			
" 15	8.0° +C.			
" 16	9.4° +C.			
" 21	rises to 11.0° +C.			
		Summer.		
Aug. 13	12.5° C.	max. 32.7° C. min. 21.1° C.	Crest	2.20
" 14	12.5° C.			
" 15	12.5° C.			
" 16	12.6° C.			
" 17	12.6° C.			

Air Currents. — The air in the cave is in almost constant motion. In general it flows up during the winter and down during the summer months. However, during the spring and autumn, the current reverses several times before the constant direction of the extreme season following is assumed. The direction of the current was down on October 14, and reversed on October 21, 22, 23, 26 and 30. After December 10 the air moved upward until the unstable period of spring began, which occurred about the end of March.

The rate of the current varies in different parts of the cave, being scarcely perceptible in the large rooms and very marked where the cave is small in cross-section. Observations taken at a single point indicate that the rate of the air current varies directly with the divergence of the subterranean and surface temperatures. Rate of air currents was measured with a meter recording a minimum of 30 feet per minute.

The upward moving current of cold air weathers the rocks in the regions of the lower openings of the cave, giving them in general a funnel shape. In this enlarged region there is always an upper stratum of air moving in the direction opposite to that of the primary cave air current.

The closed passages near Shawnee entrance have the ordinary

convection of a closed room, *i. e.*, a lower and an upper current moving in opposite directions. When the air currents were too weak to be measured, their direction was observed by means of a candle flame.

These results confirm and elaborate the results of Banta ('07) and Egli ('04). Banta found by weekly observations in Mayfield's Cave that the air currents reversed about October 1 and April 1. Egli found in Höll-Loch, a cavern explored by him, that the currents were down when the outer temperature was 8.1° Centigrade, and up when 2.3° Centigrade or below, being very strong at — 8° Centigrade.

Method. — It is much more difficult to collect plankton in a cave than in ordinary waters. The lack of light, the dimensions of pools, the relatively small amount of plankton, the great and sudden variations in the cave stream, and the necessity of transporting and manipulating the apparatus unassisted, rendered many of the ordinary methods impracticable.

After various experiments the method finally adopted for all quantitative work was as follows : A net was constructed of bolting silk No. 20 (Dufour) after the pattern described by Kofoid ('97). The net was suspended in a pool with about 3 inches of its filtering surface exposed. Two hundred gallons of water were dipped and poured into it with a bucket (1 ½ gal. capacity) at the rate of 10 gals. per minute. The catch was preserved in a 4 per cent. solution of formalin. I am well aware that this method results in some error (Kofoid, '97), but it was the most practicable for this investigation.

For the examination of material, glass troughs were constructed by cementing glass strips on a slide, with marine glue. The width approximated the diameter of the field of the microscope (⅔ obj., 2 in. ocu.), the length 40 mm., and the depth about 1 mm. The material was pipetted into this trough until the surface film was parallel to the slide. The entire catch was counted in quantitative work.

When higher powers of the microscope were necessary for careful study, the material was removed from this trough to a slide on which strips of cover glass were glued for the support of the cover glass. After examination, the material was washed from the trough into a new vial with 4 per cent. formalin.

Amount of the Plankton. — The amount of plankton is small when compared with the amount taken in a lake or epigean stream. In low stages, 200 gallons contained less than 1 c.c. In times of flood the catch was increased very much (200 c.c. or 300 c.c.), but this increase was largely due to silt and vegetable débris carried in suspension.

Constituents of the Plankton. — No organisms were found that were not referable to known epigean forms. Rather marked variations from the type were observed in some of the crustacea, notably in the genus *Bosmina*. However, the recent work of Wesenberg-Lund makes it advisable to withhold judgment on this point, until the local and seasonal variations in the crustacea of the ponds of the region have been investigated.

The zoöplanktonts taken included Copepoda, Cladocera, Rotifera and Protozoa.

The phytoplanktonts belonged to the following genera : *Spirogyra*, *Zygnema*, *Volvox*, *Closterium*, *Micriasteria*, *Vaucheria* and *Pandorina*. In addition to these, diatoms, worms, insect larvæ and fragments of spiders occurred rarely.

The planktonts having the widest temporal distribution were *Bosmina cornuta*, *Cyclops bicuspidatus* and *Anura cochlearis*. *Cyclops prasinus* outnumbered *Cyclops bicuspidatus* in November and several species of Rotifera were quite common in May.

The loricum probably enables rotifers to withstand cave conditions as all the Rotifera except two belong to the suborder Loricata. These two were found when the current was quite rapid and disappeared when the water became low and the current reduced. There may be other factors in the organization of the Loricata besides the loricum that enables them to exist under these conditions. That the rotifer fauna of the cave is included with rare exceptions in this subclass is certainly significant.

Diatoms were very rare. This was probably due to two things — (1) these forms live on the bottom of the ponds and consequently few are carried into the cave, (2) they soon succumb in the cave.

Other algæ were never taken in large quantity, but *Volvox* and *Closterium* were present in most of the catches throughout the year. Filamentous algæ were found at Shawnee Cave only in the higher stages of the stream.

The Source of the Cave Plankton. — To understand the distribution of the cave plankton, it is necessary to understand its source. It has been assumed that the plankton (at least in part) inhabiting a cave stream has been derived from the surface through sink-holes.[1] An examination of the local sink-holes made it clear that probably only a small part of the total number contribute.

Sink-holes may be divided into two principal classes, compound sink-holes and simple sink-holes. A compound sink-hole is a large depression having secondary sinks on its slopes. These arise in most cases probably by the reduction of the divides of the compound sinks by erosion. Sinks may arise secondarily on the slope of an old one.

A simple sink-hole is one without secondary sinks. Simple sink-holes agree in having the form of an inverted cone and are of three types. The first type has an opening at the apex of the cone leading to an underground passage.

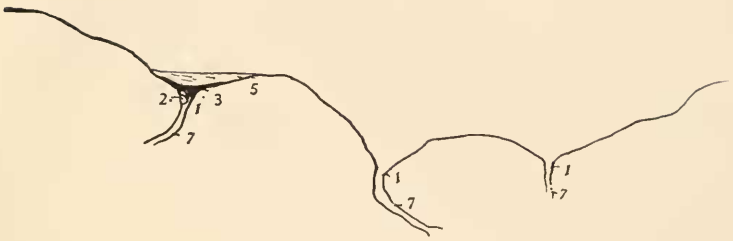
The second type has this opening closed. This closure is inaugurated by rocks and earth caving in from the sides of the opening. Sediment composed of the fine clay from the sides is then deposited over this, and forms a very impermeable layer. This results in a more or less permanent pond.

The third type of simple sink-holes is like the second, except that a new opening to a subterranean passage has been formed on its sides a short distance above its lowest point. This new opening is formed from below, but the details of the process I could not determine. The result is a pond, which at its higher levels overflows into the new opening.

Open sink-holes are the rule in land covered with timber, because the erosion in such areas occurs slowly.

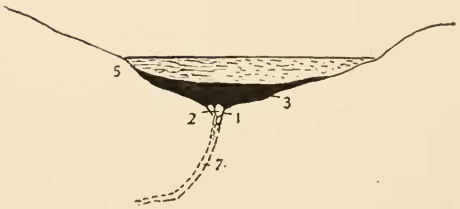
The plankton is derived from the simple sink-holes of the third type and compound sink-holes in which the lower sinks are open and the upper are closed, and in which the divides are low enough to allow the upper ones to overflow into the lower. The first type of simple sink-holes allows the water to flow so quickly into the underground channel, that organisms do not have time

[1] The cave region is a plateau which has not yet developed surface streams. The surface of this region is drained by funnel-shaped depressions called "sink-holes."

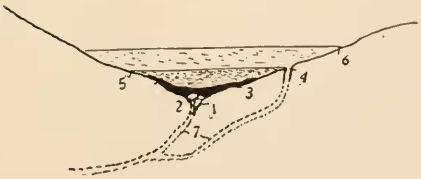


TYPE I.

TYPE II.



TYPE III.



Diagrammatic section of compound and simple sink holes. 1, original opening; 2, obstruction; 3, impervious layer of clay; 4, second opening; 5, ordinary water-level; 6, flood water-level; 7, subterranean passage.

to develop. A few wind-blown eggs and cysts may be carried into the cave and develop there, but I think it extremely doubtful. The second type of simple sinks develops many organisms, but they are not admitted to the cave stream.

Temporal Distributions. — The temporal distribution of the plankton in this cave is puzzling and the causes of this distribution are so complex that their analysis is difficult.

Although qualitative methods only were used during the fall and winter, there was less plankton in early winter than in the autumn. From this it was tentatively concluded that the main outlines of the temporal distribution of the cave plankton coincide with that of lakes and rivers in temperate latitudes, in having spring and autumnal maxima, and summer and winter minima.

After a rain of 4.71 inches which fell on February 13 and 14, I was unable to detect any organisms in the catch. Possibly a few were present and were overlooked because of the great amount of silt.

In March a series of quantitative collections was begun and continued for six months. During this period, two maxima occurred. One was on May 14, and the other was on August 13. These were maxima both in number of individuals and in number of species. Each was preceded by a very heavy rain. Between these maxima there were no rains sufficient to affect the volume of the cave stream. The maximum of May was larger than that of August (see Table II).

The February flood was accompanied by a marked decrease, but those of May and August were accompanied by a sudden increase and followed by a gradual decrease.

From these facts it is safe to conclude that excessive rainfall influences the amount of plankton per gallon of water in the cave, but not always in the same way. Excessive rainfall affects the amount of plankton in three ways. (1) The pools in plankton producing sinks of the land surface over the cave overflow and the organisms contained in them are carried into the cave. (2) The plankton is diluted in the sink where it is produced and is further diluted in the cave by the water from sink-holes of the first type. (3) The stream level is raised and the current is increased This increase in the current causes many organisms to

Table II.

Showing Influence of Flood upon Temporal Distribution.

Date.	Class or Genera.					Rainfall in Inches for Week Ending
	Cyclops.	Nauplii of *Cyclops.*	Rotifera.	*Difflugia.*	*Bosmina.*	
March 24	85	63	28	5	0	.53
" 30	75	93	74	6	0	.89
April 7	44	50	42	6	1	1.26
" 14	29	20	12	6	1	1.13
" 21	14	12	15	6	3	.32
" 28	11	46	18	3	1	.31
May 7	count incomplete on account of large amount of silt.					5.46
" 14	410	319	717	21	1998	.00
" 26	85	47	24	30	32	.85
June 2	34	30	20	16	6	.00
" 9	10	10	5	4	5	.66
" 16	11	23	14	2	6	.17
" 23	22	55	2	7	0	.00
" 30	17	42	3	10	0	.20
July 7	9	31	1	4	1	.12
" 14	28	139	3	18	0	.00
" 21	21	92	3	2	0	.15
" 30	18	84	15	5	15	.85
Aug. 7	277	325	32	34	38	1.85

be carried through the cave that would succumb to the cave environment at ordinary stream levels. The first and third of these effects of excessive rainfall are therefore positive and the second negative.

The cycle of pond life in this region is not well known, but is being investigated. It is very probable that in its main outlines it resembles that of lakes in the same latitude.

The minimum of February was due to two things, the small number of organisms produced in the pond and the great dilution by rain water.

The increase in the amount of plankton after the heavy rains of May and August was due to the fact that the amount contributed was so great that the dilution was insufficient to reduce it to the amount per gallon previously present in the cave. The increase in the number of species was caused by the rapid current carrying through many of the forms that would have succumbed to the destructive influences of the cave environment at lower stages of the water. The subsequent gradual decrease in amount and number of species was due to the destructive cave environment.

The temporal distribution of the plankton in this cave depends

upon : (1) the production of the plankton in the ponds, (2) the overflow of the ponds into the subterranean passages, (3) the dilution by rain water and interstratal seepage, (4) the rate of current in the cave stream, (5) the ability of the planktonts to live in the cave environment.

Local Distribution. — Observations taken at Dalton Cave and at Shawnee Cave indicate that the amount of plankton in the lower portion of the cave is less than in the upper portion, the amount at Shawnee varying from 40 per cent. to 80 per cent. of the amount at Upper Dalton. This is due, I think, principally to the destructive influence of the cave environment (see Table III.).

TABLE III.

Genus or Class.	Number per 200 gal. at Upper Dalton Cave.	Number per 200 gal. at Shawnee Cave.
Cyclops	59	41
Nauplii of *Cyclops*	89	64
Daphnia (immature)	0	1
Difflugia	11	3
Arcella	1	0
Œdogonium	1	0
Pandorina	2	0
Rotifera	2	4
Total	165	113

Feeding and Reproduction of the Plankton in the Cave. — The alimentary tracts of the crustacea always contained some food. In the alimentary tracts of *Cyclops* and *Bosmina* at ordinary stages of the cave stream, there was nearly as much food as in members of these genera taken above ground. In the lower stages of the stream the amount of food contained decreased.

Eggs were common in the egg sack of *Cyclops*, in the brood chambers of *Bosmina*, and upon *Anura cochlearis*. They were also observed in *Daphnia*, but the individuals of this genus were rare.

Nauplii of *Cyclops* were common throughout the year and the young of *Daphnia* and *Bosmina* were observed.

It is evident that some of the planktonts are able to continue their nutritive and reproductive processes under cave conditions, although this environment inhibits them. Whether these forms

would be able to maintain themselves in a permanent pool cut off from the cave stream is conjectural, as no such pool exists in this cave.

Tha the algæ could not live in the total darkness of the cave is certain. The method was not adapted to the investigation of the bacteria and the infusoria.

Relation of the Cave Plankton to the Permanent Cave Fauna.— The plankton of this cave does not form nor can it become a part of the permanent fauna of the cave, because the current of the stream in its higher stages is powerful enough to carry out of the cave all forms that are not strong swimmers, or have not developed the habit of living under rocks or on the bottom. *Cæcidotea*, *Crangonyx* and *Cambarus* live on the bottom and under rocks. *Amblyopsis* is a fairly strong swimmer and when struck by a current goes to the bottom or under a rock. These animals are examples of forms whose habits prevent them from being carried out of the cave.

Banta has taken *Cyclops* from the stomach of *Amblyopsis spelæus*. This suggests that the plankton is a source of food for some of the permanent cave animals.

The organisms of the cave plankton are essentially pond forms and their presence in the cave is accidental. They do not migrate into the cave to colonize it.

Summary and Conclusions. — 1. The form of the cave is determined by the direction of the seams in the limestone, the obstructions, and a factor X, which is probably elevation.

2. The form of the cave thus determined results in a large amount of residual water in the cave.

3. This residual water causes the extreme flood temperature to occur twenty-four hours or more after the crest of the flood. It also causes the rate at which the plankton is carried through the cave to vary directly as the stream level varies.

4. Water temperature varies much more than the air temperature in the interior of the cave.

5. Air currents in this cave are caused by and their rate varies directly with the divergence of terranean and subterranean temperature.

6. The amount of plankton in the cave is relatively small.

7. The plankton is composed of epigean forms and is derived from ponds in such sink-holes as have an opening above their lowest points.

8. Temporal distribution of the plankton depends upon three principal factors, the production of the plankton in the pools, excessive rainfall, and the ability of the planktons to withstand cave conditions.

9. At ordinary stages of the stream more plankton occurs in the upper part of the cave than in the lower part.

10. Some of the planktonts feed and reproduce in the cave, but these processes are more or less inhibited.

11. The plankton is not a part of the permanent cave fauna but is essentially a pond fauna accidentally carried into the cave. It is either carried through the cave or perishes in it.

LIST OF SPECIES.[1]

Protozoa.

Arcella vulgaris Ehrenberg.
Difflugia globosa Dujardin.
D. pyriformis Perty.
D. lobostoma Leidy.
D. acuminata Ehrenberg.
Euglena sp.

Arthropoda.

Cyclops bicuspidatus Claus.
C. prasinus Fischer.
C. viridus Jurine.
C. serrulatus Fischer.
C. edax Forbes.
Canthocamptus sp.
Daphnia pulex DeGeer.
Ciriodaphnia consors Birge.
Bosmina bohemica Hellick. (?)
B. cornuta Jurine.
Cydorus sphæricus Mueller.

[1] Diatoms were taken occasionally. The collections sometimes contained spiders which doubtless had fallen from the walls of the cave. One round worm and two segmented worms were observed during the year.

Pleuroxus hamatus Birge.
Cypris sp.
Campodea staphylinus (?) Westwood.
Larvæ of diptera and coleoptera rarely.

Trochelminthes.

Asplanchna ebbesborni Hudson.
Triarthra longisetæ Ehrenberg.
Cathypna luna Ehrenberg.
Monastyla lunaris Ehrenberg.
M. bulla Gosse.
Branchionis militaris Ehrenberg.
B. bakeri Ehrenberg.
Noteus quadricornus Ehrenberg.
Anura cochlearis Gosse.
Notholca longispina Kellicott.

Algæ.

Œdogonium sp.
Cladophora sp.
Vaucheria sp.
Spirogyra sp.
Zygnema sp.
Closterium subcostatum Nord.
C. lanceolatum Kg.
Cosmarium sp.
Micrasterias sp.
Volvox globator Ehrenberg.
V. aureus Ehrenberg.
Pandorina morum Bory.
Pleurococcus sp.
Pediastrum boryanum Menegh.
Senedesmus sp.
Sphærocystis schrœteri Chod.
Nostoc minutissimum Kg.
Oscillatoria sp.

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Explanation of Map.

Shawnee Cave (the outlet). Sec. I., No. 1.
Closed chamber caused by collapse of roof at Sec. I., Nos. 2–3.
Cascade. Sec. I., No. 6.
Double passage. Sec. I., Nos. 7–8.
Old cross cave. Sec. I., Nos. 9–10.
New passages. Sec. I., Nos. 1–8 and 11–13.
Opening in roof leading to *upper older* levels of cave. Sec. I., No. 14.
"Big Room." Sec. I., Nos. 15, 16, 17, 18, 19, 20, 21, 22.
"Fallen Rock." Sec. I., No. 31.
Lower Twin Cave. Sec. I., No. 32.
Upper Twin Cave. Sec. I., No. 33.
Roof too low for passage of boat. Sec. I., No. 34.
Deepest water in cave, 10 feet 4 inches. Sec. I., No. 35.
Lower Dalton Cave. Sec. I., No. 36.
Upper Dalton Cave. Sec. I., No. 37.

FIG. 1. Map of Shawnee Cave, section 1, from Shawnee to Lower Dalton. Length 4,453 feet. Scale 200 feet to the inch.

Explanation of Map.

Upper Dalton Cave. Sec. II., No. 37.
"Cross bedding" in limestone. Sec. II., Nos. 46–47.
"Old passages." Sec. II,, Nos. 56–57.
Obstruction past which boat cannot be taken. Sec. II., No. 63.
End of exploration. Sec. II., No. 64.

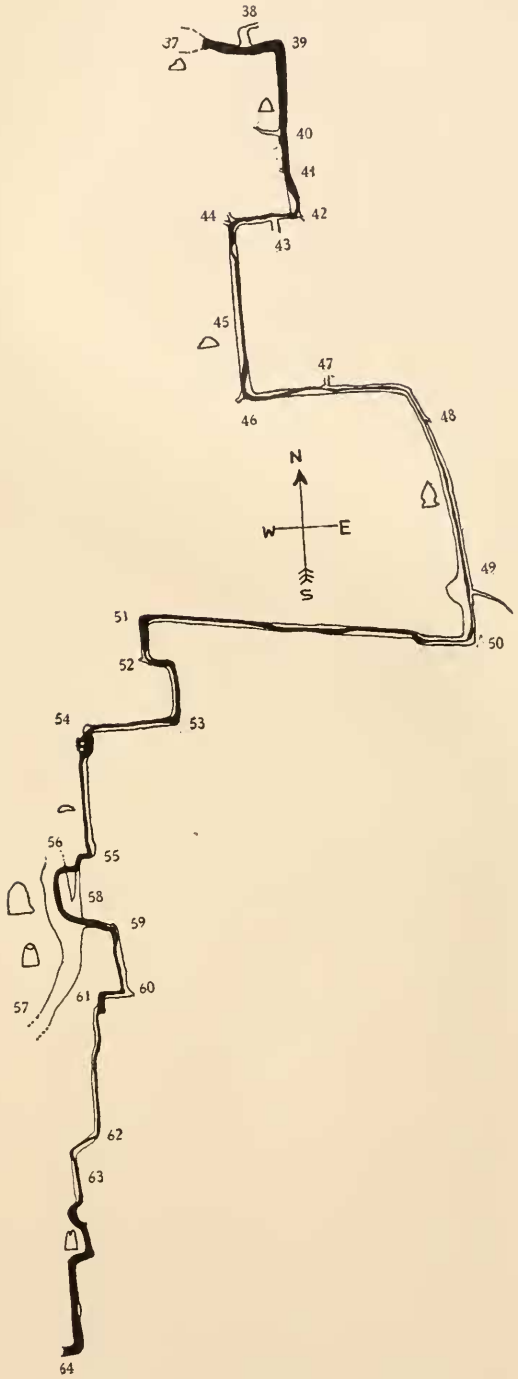

FIG. 2. Map of Shawnee Cave, section 2, from Lower Dalton to unexplored part. Length 4,674 feet. Scale 200 feet to the inch.